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(71) Applicant: SERPIN PHARMA, LLC [US/US]; 14645
Sulky Run Ct., Nokesville, VA 20181 (US).(72) Inventor: GELBER, Cohava; 14645 Sulky Run Ct.,
Nokesville, VA 20181 (US).(74) Agent: HOLMES-SON, Michelle; Mintz Levin Cohn Fer-
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(54) Title: METHODS FOR TREATMENT OF DISEASES

SERPIN C-TERMINAL PEPTIDES

HUMAN AAT C36	1	S	I	P	P	E	V	K	F	N	K	P	F	V	F	L	M	I	E	Q	N	T	K	S	P	L	F	M	G	K	V	V	N	P	T	Q	K			
HUMAN KALLIST ATIN (C39)	2	S	A	Q	T	N	R	H	I	L	R	F	N	R	P	F	L	V	V	L	F	S	T	S	T	Q	S	V	L	F	L	G	K	V	V	D	P	T	K	P
HUMAN ANTICHY MOTRYPSI n (C40)	3	S	A	L	V	E	T	R	T	I	V	R	F	N	R	P	F	L	M	I	V	P	T	D	T	Q	N	I	F	F	M	S	K	V	T	N	P	K	Q	A
RAT SERPINA3 M (C38)	4	S	G	R	P	P	M	I	V	W	F	N	R	P	F	L	I	A	V	S	H	T	H	G	Q	T	I	L	F	M	A	K	V	I	N	P	V	G	A	
RAT SERPINA3 K (C38)	5	K	S	L	P	Q	T	I	P	L	L	N	F	N	R	P	F	M	L	V	I	T	D	D	N	G	Q	S	V	F	F	M	G	K	V	T	N	P	M	
HUMAN HYBRID 1	6	S	A	Q	V	E	T	I	V	R	F	N	R	P	F	L	V	I	V	S	T	N	T	Q																
HUMAN- RAT HYBRID 1	7	S	I	P	P	Q	M	I	V	W	F	N	R	P	F	L	I	A	S	H	T	H	T	Q																

FIG. 1

(57) Abstract: We describe peptides and their uses for the treatment of, e.g. acute myocardial infarction and other cytokine storm-associated conditions.

METHODS FOR TREATMENT OF DISEASES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of priority under 35 U.S.C. §119(e) to provisional patent application serial number 62/211,296 filed August 28, 2015, the contents of which are incorporated herein by reference in their entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing, which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on December 31, 2012, is named 65292741.txt and is 18,884 bytes in size.

FIELD OF THE INVENTION

[0003] The present disclosure presents novel methods for treatment of diseases, including acute myocardial infarction (AMI); gout; ischemia; stroke; traumatic brain injury; toxic shock, and heart surgery complications using isolated and/or synthesized peptides having unexpected cytoprotective properties.

BACKGROUND

[0004] Acute myocardial infarction (AMI) remains a major cause of morbidity and mortality in the US and worldwide. Despite current strategies for early reperfusion, many patients die early during the course, and those who survive are at risk for dying later from adverse cardiac remodeling, heart failure, and sudden death.

[0005] Numerous publications emphasize the correlation between the size of the infarct in AMI and the probability to progress to heart failure in the next 2-5 years post AMI.

[0006] Patients presenting with ST-segment elevation (STEMI) are at particularly high risk for adverse cardiac remodeling, heart failure, and in-hospital and long-term mortality. Although there have been considerable improvements in the treatment of STEMI, the reduction in early mortality has been associated with an increasing incidence of heart failure after STEMI. This likely reflects more high risk patients surviving the index event as well as the aging of the population and the epidemics of hypertension and diabetes. Within 30 days of

STEMI, more than 20% of survivors are diagnosed with heart failure, a disease associated with high morbidity, disability, and mortality.

[0007] Heart failure is indeed a major public health problem affecting approximately 5 million Americans with 500,000 new cases per year. In contrast to other cardiovascular disease, the incidence and prevalence of heart failure continue to increase and heart failure is now the leading cause of hospitalization for people aged 65 years, a segment of the population that is also rapidly growing. Although survival after the onset of heart failure is also improved, current therapies may slow but not halt the progression of the disease. With the limitations to functional capacity, the progressive symptoms of dyspnea and fatigue, the frequent hospital admissions and the economic consequences of lost productivity and increasing costs of medical care, heart failure imposes a significant burden on healthcare.

[0008] There is an urgent need to develop additional treatments to minimize the infarct size and prevent heart failure after AMI. The current treatment in STEMI includes prompt reperfusion of the ischemic myocardium by restoration of the coronary artery patency (i.e. angioplasty or fibrinolysis), prevention of re-occlusion (i.e. antiplatelet and anticoagulants), and neuro-hormonal blockade (i.e. renin-angiotensin-aldosterone and adrenergic blockers). While each of these interventions provide incremental benefit and significantly reduce morbidity and mortality, the incidence of heart failure after STEMI has continued to rise, implying that the current treatment paradigm still misses one or more key pathophysiologic mechanisms

[0009] Determining the mechanisms by which unfavorable cardiac remodeling and heart failure progress despite optimal treatment is thus a critical step in the search for novel interventions, with the ultimate goal of reducing the incidence, burden, and mortality of heart failure after STEMI.

SUMMARY

[0010] We have unexpectedly found that C-terminal peptides from the Serpin molecule (e.g., short peptide, SP16 (SEQ ID NO: 1)) and variants and derivatives thereof function as potent cytoprotective agents in the treatment of conditions associated with cytokine storms and/or infarctions, such as AMI. We have previously shown that these peptides have anti-inflammatory properties (see, e.g., U.S. Patent No. 8,975,224), which makes their efficacy in treating conditions associated cell death due to cytokine storms and/or infarctions surprising. In the relevant conditions, while uncontrolled inflammation can contribute to the disease

process, inflammation is none the less a critical process which is required for recovery. This is demonstrated by the fact that many attempts to modulate the inflammatory response as a treatment for, e.g., acute myocardial infarction (AMI) have failed.

[0011] In contrast, and surprisingly, the peptides described herein 1) permit therapeutic modulation of the inflammatory response, 2) exert a cytoprotective effect, and 3) reduce infarct size. This combination of activities provides a surprising, and unexpected efficacy in treating conditions associated with cytokine storms and/or infarctions. As described in the examples herein, this surprising efficacy is contrasted with the merely anti-inflammatory activity of, e.g., an IL-1 antagonist.

[0012] Accordingly, we provide peptide compositions, pharmaceutical compositions comprising the C-terminal Serpin peptides and methods of using the peptides to treat conditions including cytokine-storm associated conditions and/or to reduce infarctions occurring in a number of conditions, where cytoprotection is desired, such as in conditions associated with a risk for ischemia reperfusion injury and resulting pathologies. The unexpected cytoprotective properties of these peptides allow use of the compositions comprising such peptides to new indications, and allow preventive intervention in conditions associated with, e.g., ischemia reperfusion injury.

[0013] Specifically, we have shown the novel cytoprotective function of the SP16 peptide consisting of an amino acid sequence VKFNKPFVFLMIEQNTK (SEQ ID NO: 1) or SP163M VKFNKPFVFLNleIEQNTK in well-established animal models for acute myocardial infarction (AMI).

[0014] Accordingly, we provide novel uses for a composition comprising an isolated peptide comprising, consisting essentially of, or consisting of the amino acid sequence X1-Z1-F-N-K -P-F-X2-Z2-X3-Z3-Q (SEQ ID NO: 2), wherein

[0015] X1 is V or L;

[0016] X2 is V, L or M or Nle;

[0017] X3 is M, Nle, I or V;

[0018] Z1 is any amino acid;

[0019] Z2 is a sequence of any two amino acids; and

[0020] Z3 is a sequence any five amino acids, and wherein the isolated peptide consists of 37 or fewer amino acids.

[0021] The peptide can be modified to extend the shelf life and/or bioavailability using one or more non-natural peptide bonds or amino acids or by attaching to the peptide functional groups such as, e.g., polyethylene glycol (PEG).

[0022] The composition may further comprise a carrier, such as a pharmaceutically acceptable carrier.

[0023] In one aspect of any of the embodiments described herein is a method of 1) treating a disease associated with a cytokine storm, 2) reducing an infarct size, and/or 3) treating acute myocardial infarction (AMI), the method, comprising administering to a human subject affected with the disease a pharmaceutical composition comprising a peptide selected from the group consisting of:

- (a) a peptide comprising the amino acid sequence
X1-Z1-F-N-K-P-F-X2-Z2-X3-Z3-Q (SEQ ID NO: 2), wherein
X1 is V or L;
X2 is V, L, Nle, or M;
X3 is M, Nle, I or V;
Z1 is any amino acid;
Z2 is a sequence of any two amino acids; and
Z3 is a sequence any five amino acids, and wherein the peptide comprises 37 or fewer amino acids;
- (b) a peptide comprising the amino acid sequence VKFNKPFVFLMIEQNTK
(SEQ ID NO: 1);
- (c) a peptide consisting essentially of the amino acid sequence
X1-Z1-F-N-X2-P-F-X3-Z2-X4-Z3-X5 (SEQ ID NO: 3), wherein
X1 is V or L;
X2 is K or R;
X3 is V, L, Nle, or M;
X4 is M, Nle, I or V;
X5 is K or Q;
Z1 is any amino acid;
Z2 is a sequence of any two amino acids; and
Z3 is a sequence any five amino acids;

- (d) a peptide consisting essentially of the amino acid sequence RFNRPFLR (SEQ ID NO: 4).
- (e) a peptide consisting essentially of the amino acid sequence of RRRFNRPFLRRR (SEQ ID NO: 8).
- (f) a peptide consisting essentially of the amino acid sequence of VKFNKPFVFLMIEQNTK (SEQ ID NO: 1);
- (g) a peptide consisting essentially of the amino acid sequence of FNRPFLL (SEQ ID NO: 10); and
- (h) a peptide comprising SEQ ID NO: 57.

[0024] In some embodiments of any of the aspects described herein, the disease associated with a cytokine storm is selected from the group consisting of acute myocardial infarction (AMI); gout; stroke; heart surgery complications; traumatic brain injury; acute respiratory distress syndrome (ARDS), sepsis, Ebola, avian influenza, smallpox, and systemic inflammatory response syndrome (SIRS). In some embodiments of any of the aspects described herein, the human subject in need of a reduction in infarct size is a subject in need of treatment for a disease selected from the group consisting of: acute myocardial infarction (AMI); ischemia; stroke; traumatic brain injury; and toxic shock.

[0025] In some embodiments of any of the aspects described herein, the pharmaceutical composition is an oral formulation.

[0026] In some embodiments of any of the aspects described herein, the peptide further comprises at least one second peptide or protein. In some embodiments of any of the aspects described herein, the at least one second protein or peptide is attached to the peptide as a fusion peptide. In some embodiments of any of the aspects described herein, the at least one second peptide or protein is an epitope tag or a half-life extender or both. In some embodiments of any of the aspects described herein, the peptide comprises one or more D-amino acids.

[0027] In some embodiments of any of the aspects described herein, the peptide causes a 75% decrease in serum TNF- α levels when administered in an effective amount to a human subject.

[0028] In some embodiments of any of the aspects described herein, the peptide consists of 35 amino acid residues or fewer. In some embodiments of any of the aspects described herein, the peptide consists of 22 amino acid residues or fewer. In some embodiments of any of the aspects described herein, the peptide consists of 21 amino acid residues or fewer.

[0029] In some embodiments of any of the aspects described herein, the composition further comprises a pharmaceutically acceptable carrier.

[0030] In some embodiments of any of the aspects described herein, the subject does not have, does not have symptoms of, or is not diagnosed as having a condition selected from the group consisting of: type II diabetes, lupus, graft versus host disease, uveitis, eczema, psoriasis, cystic fibrosis, rheumatoid arthritis, acute radiation syndrome, burn patients, inflammatory bowel disease, type I diabetes, and hyperglycemia.

[0031] The peptides of the invention can be used to reduce the serum TNF- α levels in human individuals who have pathologically increased TNF- α levels. Thus the invention provides a method or use for reducing TNF- α levels in a human in need thereof comprising administering to the human individual the peptide of the invention in a pharmaceutically acceptable carrier. In certain embodiments, the isolated peptide results in a 50% or 75% decrease in serum TNF- α levels when administered in an effective amount to a human subject compared to the levels before administration of the isolated polypeptide. In other embodiments, the isolated peptide further comprises at least one other protein. The combination of the at least two proteins can be referred to as a fusion protein. The other protein can be selected from an epitope tag and a half-life extender. The peptide can comprise both an epitope tag and a half-life extender.

[0032] We also provides a composition comprising an isolated peptide consisting essentially of or consisting of the amino acid sequence X1-Z1-F-N-X2-P-F-X3-Z2-X4-Z3-X5 (SEQ ID NO: 3), wherein

[0033] X1 is V or L;

[0034] X2 is K or R;

[0035] X3 is V, L M, or Nle;

[0036] X4 is M, Nle, I or V;

[0037] X5 is K or Q;

[0038] Z1 is any amino acid;

[0039] Z2 is a sequence of any two amino acids;

[0040] Z3 is a sequence any five amino acids; and

[0041] wherein the isolated peptide causes a 75% decrease in serum TNF- α levels when administered in an effective amount to a human subject compared to the amount of TNF- α levels before administering the peptide.

[0042] In certain embodiments, the isolated peptide comprises the amino acid sequence X1-Z1-F-N-X2-P-F-X3-Z2-X4-Z3-X5 (SEQ ID NO: 3), wherein X1, X2, X3, X4, X5, Z1, Z2, and Z3 are defined as above and wherein the peptide consists of, at most, 35, 22 or 21 amino acid residues.

[0043] In certain aspects, the peptides of any of the embodiments described herein and throughout the specification, also comprise at least one other protein. The combination of these at least two proteins can be referred to as a fusion protein. Specifically the other protein can be selected from an epitope tag and a half-life extender. In some aspects of all the embodiments of the invention, the isolated peptide can comprise both an epitope tag and a half-life extender.

[0044] The disclosure also provides an isolated peptide consisting essentially of or consisting of the amino acid sequence RFNRPFLR (SEQ ID NO: 4) and RFNKPFLR (SEQ ID NO: 5). In certain embodiments, the isolated peptide causes a 50% or 75% decrease in serum TNF- α levels compared to the amount of TNF- α levels before administering the peptide when administered in an effective amount to a human subject.

[0045] In other aspects of all the embodiments of the invention, the isolated peptide is linked another protein. The combination of these proteins can be referred to as a fusion protein. Specifically the other protein can be selected an epitope tag and a half-life extender.

[0046] In some aspects of all the embodiments of the invention, the isolated peptide consists of, at most, 100, 35, 22, 21 or 9 additional amino acids.

[0047] In other embodiments, the isolated peptide consists essentially of, or consists of the amino acid sequence of Z1-RFNRPFLR-Z2 (SEQ ID NO: 6) and Z1-RFNKPFLR-Z2 (SEQ ID NO: 7), wherein Z1 and Z2 are independently 1, 2, 3, 4, 5, 6, 6, 7, 8, 9, 10 or between 1 and 3, between 1 and 5, between 1 and 6, between 1 and 7, between 1 and 8, between 1 and 9, or between 1 and 10 basic amino acids.

[0048] In some embodiments, the isolated peptide consists essentially of or consists of the amino acid sequence of RRRFNRPFLRRR (SEQ ID NO: 8) and RRRFNKPFLRRR (SEQ ID NO: 9).

[0049] The disclosure also provides a composition comprising an isolated peptide consisting essentially of or consisting of the amino acid sequence of FNRPFLL (SEQ ID NO: 10) and FNKPFL (SEQ ID NO: 11).

[0050] The disclosure also provide a composition comprising an isolated or synthesized peptide consisting essentially of or consisting of any one or a combination of the following

peptides: SP40; SP43; SP46; and SP49 as set forth in Table B, and their use in methods for 1) treating a disease associated with a cytokine storm, 2) reducing an infarct size, and/or 3) treating acute myocardial infarction (AMI).

[0051] In some embodiments of any of the aspects described herein, the disease associated with a cytokine storm is selected from the group consisting of acute myocardial infarction (AMI); gout; stroke; heart surgery complications; traumatic brain injury; acute respiratory distress syndrome (ARDS), sepsis, Ebola, avian influenza, smallpox, and systemic inflammatory response syndrome (SIRS). In some embodiments of any of the aspects described herein, the human subject in need of a reduction in infarct size is a subject in need of treatment for a disease selected from the group consisting of: acute myocardial infarction (AMI); ischemia; stroke; traumatic brain injury; and toxic shock.

[0052] In one embodiment, the disclosure also provides a method of decreasing serum TNF- α compared to the amount of TNF- α levels before administering the peptide to a subject comprising administering to the subject an effective amount of any one of the isolated peptides as defined above to decrease the serum TNF- α levels by at least 50%. In one embodiment, serum TNF- α levels are decreased by 75% compared to the amount of TNF- α levels before administering the peptide. In other embodiments, the subject is a mammal. In some aspects of all the embodiments of the invention, the mammal is a human.

[0053] In some aspects of all the embodiments of the invention, the human has been diagnosed with a disease associated with a cytokine storm, AMI, or an infarct prior to administering the peptide. In some aspects of all the embodiments of the invention, the human has been diagnosed with acute myocardial infarction (AMI); gout, stroke, heart surgery complications, traumatic brain injury, acute respiratory distress syndrome (ARDS), sepsis, Ebola, avian influenza, smallpox, systemic inflammatory response syndrome (SIRS), ischemia, and/or toxic shock prior to administering the peptide.

[0054] In some aspects, the human has not been subjected to prior treatment with alpha antitrypsin, such as alpha-1-antitrypsin treatment before the treatment with the peptides of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0055] This patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0056] **Figure 1** is a table showing the sequences and homology of Serpin C-terminal peptides (SEQ ID NOs: 18-24, respectively, in order of appearance).

[0057] **Figure 2** is a table showing peptides derived from truncations (SEQ ID NOs: 25-32, 1, 33-34, 1, 33, 38-51, 10, and 8, respectively, in order of appearance).

[0058] **Figures 3A-3D** depict graphs and images demonstrating that AAT reduces infarct size in experimental acute myocardial infarct (AMI) in the mouse. Fig. 3A depicts a graph of the area at risk in the le. ventricle. Fig. 3B depicts a graph of infarct size (% of le. ventricle). CDD. Masson's trichrome stain of a midventricular heart section from a representative control mouse treated with albumin (Fig. 3C) or AAT (Fig. 3D) 7 days after AMI. The infarct scar is evident in blue. Alb=albumin.

[0059] **Figures 4A-4C** depicts graphs demonstrating the efficacy of SP163M in reducing myocardial infarct size (Fig. 4A) reducing plasma levels of cardiac specific troponin I, a biomarker of myocardial necrosis (Fig. 4B), and , was also significantly reduced by SP16 treatment (Figure 4B), and preserving the left ventricular systolic function, measured as left ventricular fractional shortening using transthoracic-echocardiography (Figure 4C).

[0060] **Figures 5A and 5B** are graphs demonstrating that SP163M given with 30' of reperfusion significantly reduced infarct size (Figure 5A) and preservation of LV fractional shortening (Figure 5B).

[0061] **Figures 6A and 6B** are graphs demonstrating that SP163M given with 30' of reperfusion reduces infarct size (Figure 6A) and preserves LV fractional shortening (Figure 6B) in a dose-dependent fashion.

[0062] **Figure 7** is a graph demonstrating that SP163M does not affect heart contractility. Depicted are measurements of cardiac systolic function at rest (left panel) or after isoproterenol challenge (contractile reserve) (right panel).

[0063] **Figure 8** depicts images and echocardiograms demonstrating that SP163M reduces infarct size and effectively treats AMI.

[0064] **Figure 9** depicts a proposed model of the cytoprotective mechanism of action of SP16 and SP163M.

[0065] **Figure 10** depicts graphs of flow cytometry demonstrating that SP16 increases expression of LRP1 on Raw264.7 murine macrophages in a dose-dependent manner.

[0066] **Figure 11** depicts a schematic of the onset and resolution of the acute inflammatory response in acute myocardial infarction.

[0067] **Figure 12A and 12B** depict SP16 binding to LRP1. When SERPINs bind the proteases inactivating them, a conformational change occurs by which a short peptide containing a unique motif (5-11 amino acids) is exposed. Figure 12A provides a schematic (modified from Joslin G et al. J Biol Chem 1991 and Lillis A et al. Physiol Rev 2008) to illustrate that SP16 contains AAT's pentapeptide, FVFLM (amino acid residues 7-11 of SEQ ID NO:1), which is responsible for binding to LRP1. Figure 12B provides evidence that SP163M, and not SP34, binds to LRP1 *in vitro*.

[0068] **Figure 13A and 13B** provide evidence that SP16 is an LRP1 agonist. SP163M inhibits NF-kB signaling induced by LPS (Figure 13A) or Gp96 (Figure 13B) in THP1-XBlueTM-MD2-CD14 cells, and that treatment with LRP1 blocking antibody or RAP, a co-factor leading to LRP1 downregulation, limits SP16-related inhibition.

[0069] **Figure 14A and 14B** demonstrate that LRP1 mediates SP16-induced cardioprotection. Mice were pretreated with a LRP1 blocking antibody to test whether LRP1 mediated the cardioprotective signal of SP163M and AAT. Treatment with the blocking antibody eliminated the protective effects of both SP163M and plasma derived AAT. N=5-8 per group.

DETAILED DESCRIPTION

[0070] The present disclosure describes a novel and unexpected use for isolated peptides in the field of disease treatment and prevention where the peptides provide cytoprotective effects. Specifically, the invention describes novel methods for treatment of diseases, where cytoprotection is an essential component of disease treatment and/or prevention of worsening of the condition, including e.g., acute myocardial infarction (AMI); gout; ischemia; stroke; traumatic brain injury; toxic shock, and heart surgery complications using isolated and/or synthesized peptides having unexpected cytoprotective properties..

[0071] Serine protease inhibitors (Serpins) represent a large (>1000) family of protease inhibitors, present in all branches of life and involved in a multitude of physiological processes. In mammals, such as humans, Serpins are important for homeostasis and although a certain level of promiscuity exists, each Serpin has a cognate serine protease(s). For example, alpha-1-antitrypsin (AAT) and alpha-1-antichymotrypsin (ACT) inhibit inflammatory proteases such as elastase, whereas antithrombin inhibits thrombin and plays a role in coagulation.

[0072] A number of specific AAT mutations are manifested in human disease, including COPD, thrombosis and Serpinopathies (cirrhosis and dementia). Currently, a small number of human serum-derived AAT formulations are approved by the FDA for treatment of COPD. In this therapeutic approach, AAT functions as a protease inhibitor similar to endogenous AAT.

[0073] AAT is the archetypical Serpin and shares tertiary structure with other Serpins. Serpins have a ~20 amino acid (aa) exposed loop, called the reactive center loop (RCL), which serves as bait for the cognate proteases. Once the protease binds the RCL, it becomes trapped, partially unfolded and destined for degradation. The cleavage of the RCL at its P1-P1' site drives the process of protease inactivation and results in the release of a small C-terminal peptide from the Serpin molecule.

[0074] We have previously described Serpin peptide fragments and derivatives thereof that were determined to have anti-inflammatory properties (U.S. Patent No. 8,975,224).

[0075] Data presented herein, from an AMI model, demonstrate that AAT-derived peptides, e.g. SP16, not only have anti-inflammatory properties but also demonstrate surprising cytoprotective and infarct reducing activity. The cytoprotective and infarct-reducing activities was not previously known and provide significant and unexpected advantages for treatment of certain diseases with the peptides described herein. This is demonstrated, for example, by the fact that SP16 is surprisingly efficacious in treating AMI as compared to compounds with only anti-inflammatory activity (e.g. an IL-1 antagonist), providing activities not achievable with mere anti-inflammatories, resulting in significantly improved therapeutic effects.

[0076] Without wishing to be bound by theory, it is contemplated that SP16 mediates the cardiac protection described herein by interfering with inflammatory cell death (pyroptosis) caused by ischemia and reperfusion. Specifically, SP16 limits acute ischemia-reperfusion injury, limits the acute inflammatory response in the heart, reduces infarct size, prevents adverse cardiac remodeling, and prevents heart failure. A proposed model for SP16's activity is depicted in Figure 9.

[0077] This discovery leads us to provide methods of treating diseases associated with cytokine storms and/or reducing infarct size, e.g. treatment of AMI, by administering molecules from the C-terminal peptides of Serpin molecules. The molecules are to be administered in an amount effective to reduce the size of an infarct or to reduce the cytotoxic effects of cytokine storms, such as ischemia reperfusion injury.

[0078] We have discovered that SP16 (SEQ ID NO: 1) and SP163M (SEQ ID NO: 57), which is derived from human alpha-antitrypsin not only exhibits anti-inflammatory and immune-modulatory properties similar to those of the parent protein, alpha-1-antitrypsin, but also acts as a cytoprotective agent. SP16 appears to be a first-in-class peptide master switch for treatment of autoimmune, inflammatory and metabolic diseases and in addition, has a surprising effect as a cytoprotective agent. Without wishing to be bound by a theory, the peptides of the invention can provide a good safety profile, based on the good safety profile of the parent protein, alpha-1-antitrypsin. However, the peptides of the invention are far easier and thus less expensive to produce as they are much smaller than the parent protein.

[0079] We have previously shown that C-terminal peptides that result from a Serpin molecule's cleavage by one of its cognate serine proteases have a biologic function that is distinct from that of the protease inhibitor function of the parent, complete Serpin molecule. For example, the C-terminal peptides from AAT, antichymotrypsin and Kallistatin have varying degrees of anti-inflammatory effects. Based on our current research and surprising results, we now submit that therapeutic uses for these peptides can be expanded from anti-inflammatory uses to cytoprotective uses. The novel function allows treatments aimed at preventing, e.g., ischemia

[0080] The previously known functions of Serpins are related to inhibiting the function of serine protease enzymes. A few Serpins inhibit other types of proteins, and several do not have an inhibitory function.

[0081] Serpins are a large family (>1000) of Serine Protease Inhibitors that are structurally similar but functionally diverse. They are involved in a multitude of physiological processes and are critical for homeostasis in mammals. Genetic mutations in individual Serpins are manifested in different human diseases, including COPD, thrombosis and emphysema.

[0082] Each serpin with an inhibitory role is responsible for blocking the activity of one or more proteins. Serpins bind to their target proteins to prevent them from completing any further reactions. Upon binding to a target, an irreversible change in the structure of a serpin protein occurs. Certain cells recognize when a Serpin is bound to its target and clear these attached proteins from the bloodstream.

[0083] Alpha-1-antitrypsin (AAT) is the prototypical Serpin. PROLASTIN® (Talecris), ZEMAIRA® (Aventis Behring) and ARALAST® (Baxter) are human serum-derived AAT

formulations approved by the FDA for treatment of COPD. AAT is currently in clinical trials for treatment of new onset type I diabetes, graft vs. host disease and cystic fibrosis.

[0084] Researchers have identified at least 37 different serpin genes in humans. Based on our research, we submit that isolated and synthesized C-terminal fragments of the serpins proteins provide a novel source of molecules with anti-inflammatory, cytoprotective, and infarct reductive activity. Thus, we submit that the C-terminal fragments of at least the Serpins listed in Table A are useful in the methods described herein.

Table A		
Approved Symbol	Approved Name	Synonyms
<u>SERPINA1</u>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	AAT, A1A, PI1, alpha-1-antitrypsin, A1AT, alpha1AT
<u>SERPINA2</u>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 2	ATR, ARGS
<u>SERPINA3</u>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	ACT, alpha-1-antichymotrypsin
<u>SERPINA4</u>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 4	KST, KAL, KLST, kallistatin
<u>SERPINA5</u>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 5	PAI3, PROCI
<u>SERPINA6</u>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 6	
<u>SERPINA7</u>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 7	
<u>AGT</u>	angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	
<u>SERPINA9</u>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 9	CENTERIN, SERPINA11b, GCET1
<u>SERPINA10</u>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 10	PZI, ZPI
<u>SERPINA11</u>	serpin peptidase inhibitor, clade A	

Table A		
Approved Symbol	Approved Name	Synonyms
	(alpha-1 antiproteinase, antitrypsin), member 11	
<u>SERPINA12</u>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 12	OL-64, Vaspin
<u>SERPINA13</u>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 13 (pseudogene)	UNQ6121
<u>SERPINB1</u>	serpin peptidase inhibitor, clade B (ovalbumin), member 1	EI, PI2, anti-elastase
<u>SERPINB2</u>	serpin peptidase inhibitor, clade B (ovalbumin), member 2	HsT1201
<u>SERPINB3</u>	serpin peptidase inhibitor, clade B (ovalbumin), member 3	T4-A, HsT1196
<u>SERPINB4</u>	serpin peptidase inhibitor, clade B (ovalbumin), member 4	PI11, LEUPIN, SCCA-2, SCCA1
<u>SERPINB5</u>	serpin peptidase inhibitor, clade B (ovalbumin), member 5	maspin
<u>SERPINB6</u>	serpin peptidase inhibitor, clade B (ovalbumin), member 6	PTI, CAP
<u>SERPINB7</u>	serpin peptidase inhibitor, clade B (ovalbumin), member 7	MEGSIN
<u>SERPINB8</u>	serpin peptidase inhibitor, clade B (ovalbumin), member 8	CAP2
<u>SERPINB9</u>	serpin peptidase inhibitor, clade B (ovalbumin), member 9	CAP3
<u>SERPINB10</u>	serpin peptidase inhibitor, clade B (ovalbumin), member 10	bomapin
<u>SERPINB11</u>	serpin peptidase inhibitor, clade B (ovalbumin), member 11 (gene/pseudogene)	EPIPIN
<u>SERPINB12</u>	serpin peptidase inhibitor, clade B (ovalbumin), member 12	YUKOPIN
<u>SERPINB13</u>	serpin peptidase inhibitor, clade B (ovalbumin), member 13	HUR7, hurpin, headpin
<u>SERPINC1</u>	serpin peptidase inhibitor, clade C (antithrombin), member 1	ATIII, MGC22579
<u>SERPIND1</u>	serpin peptidase inhibitor, clade D	HC-II, HLS2, HC2, D22S673

Table A		
Approved Symbol	Approved Name	Synonyms
	(heparin cofactor), member 1	
<u>SERPINE1</u>	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	PAI
<u>SERPINE2</u>	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2	PN1, GDN, PNI, nexin
<u>SERPINE3</u>	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 3	
<u>SERPINF1</u>	serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1	EPC-1, PIG35
<u>SERPINF2</u>	serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 2	API, ALPHA-2-PI, A2AP, AAP
<u>SERPING1</u>	serpin peptidase inhibitor, clade G (C1 inhibitor), member 1	CIIN, C1-INH, HAE1, HAE2
<u>SERPINH1</u>	serpin peptidase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1)	HSP47, colligen
<u>SERPINI1</u>	serpin peptidase inhibitor, clade I (neuroserpin), member 1	neuroserpin
<u>SERPINI2</u>	serpin peptidase inhibitor, clade I (pancpin), member 2	PANCPIN, TSA2004, MEPI, pancpin

[0085] The peptides described in U.S. Patent No. 8,975,224 are specifically defined short isolated or synthesized C-terminal peptides based on Serpins and variants and derivatives thereof with surprisingly effective anti-inflammatory properties and with much more useful size for therapeutic applications compared to the native Serpin proteins. The isolated peptides are shown in Figures 1-2. Figure 1 shows the amino acid sequences of the C-terminal fragments of a variety of Serpins. Each peptide is marked with a SEQ ID NO: in column 2, immediately to the left of the peptide. Figure 2 shows truncations of the C-terminal fragments shown in Figure 1, as well as variants and derivatives thereof. Again, each peptide is marked with a SEQ ID NO: in column 2, immediately next to the peptide.

[0086] An alanine screen demonstrated that isolated or synthesized or modified SP16 peptides shown in Table B below are particularly effective in reducing TNF-alpha levels in a mouse model for inflammation. Specifically, in these particular fragments, the three most N-terminal and the two most C-terminal amino acids appear to play a role in the anti-inflammatory properties of the peptides as replacement of them appeared to reduce the capacity of the peptides to reduce TNF-alpha levels in a LPS challenged mouse model of sepsis (see, e.g., PCT/US13/20498; which is incorporated by reference herein in its entirety). Accordingly, in some aspects of all embodiments of the invention the peptides are selected from SP40, SP43, SP46, and SP49 the peptide sequences of which are set forth in Table B.

[0087] Table B shows peptides named SP16; SP40; SP43; SP46; and SP49 provided particularly good anti-inflammatory effect when administered to a mouse model of sepsis. SP16, SP163M, SP37, SP40, and/or SP51 bind LRP1 and are contemplated to be cytoprotective as described herein. In some embodiments, SP16, SP163M, SP37, SP40, and/or SP51 can be used in the methods described herein.

Table B	Peptide Amino Acid Sequence
SP16	V K F N K P F V F L M I E Q N T K (SEQ ID NO: 1)
SP37	A A A N K P F V F L M I E Q N T K (SEQ ID NO: 12)
SP40	V K F A A A F V F L M I E Q N T K (SEQ ID NO: 13)
SP43	V K F N K P A A A L M I E Q N T K (SEQ ID NO: 14)
SP46	V K F N K P F V F A A A E Q N T K (SEQ ID NO: 15)
SP49	V K F N K P F V F L M I A A A T K (SEQ ID NO: 16)
SP51	V K F N K P F V F L M I E Q A A A (SEQ ID NO: 17)

[0088] According to some embodiments and aspects of the invention, method of providing cytoprotective treatment comprise administering to a subject, such as a human subject, an effective cytoprotection or infarct size-reducing amount of any of isolated peptides consisting of or consisting essentially of sequences set forth in SEQ ID NOs: 8, 10, 19-34, and 38-49 can be used in the methods described herein. Any of peptides consisting of or consisting essentially of sequences set forth SEQ ID NOs: 8, 10, 19-34, and 38-49 can also be used to reduce TNF- α in a subject. In certain embodiments, the amount of TNF- α in the serum is reduced by up to 50% or more or 75% or more compared to the amount of the same in the serum prior to administering the peptide.

[0089] Table C below presents additional exemplary peptides that were used to reduce TNF-alpha levels in mice subjected to an LPS challenge. Also the peptides with capacity to reduce TNF-alpha levels as described in PCT/US13/20498; which is incorporated by reference

herein in its entirety, are contemplated for the compositions, pharmaceutical compositions and methods of use and treatment of inflammatory conditions in the present invention.

Table C (SEQ ID NOS 18-32, 1, and 33-34, respectively, in order of appearance)

SP1	Human AAT C36	S I P P E	V K F N K P F V F L M I E Q N T K S P L F M G K V V N P T Q K
SP2	Human KALLISTATIN (C39)	S A Q T N R H I L R F N R P F L V V I F S T S T Q S V L F L G K V V D P T K P	
SP3	Human Antichymotrypsin (C40)	S A L V E T R T I V R F N R P F L M I I V P T D T Q N I F F M S K V T N P K Q A	
SP4	Rat Serpina3M (C38)	S G R P P M I V W F N R P F L I A V S H T H G Q T I L F M A K V I N P V G A	
SP5	Rat Serpina3K (C38)	K S L P Q T I P L L N F N R P F M L V I T D D N G Q S V F F M G K V T N P M	
SP6	Human Hybrid 1	S A Q V E T I V R F N R P F L V I I V S T N T Q	
SP7	Human-Rat Hybrid 1	S I P P Q M I V W F N R P F L I A I S H T H T Q	
SP8	Human AAT C36	S I P P E	V K F N K P F V F L M I E Q N T K
SP9	Human KALLISTATIN (C39)	S A Q T N R H I L R F N R P F L V V I F S T S T Q	
SP10	Human Antichymotrypsin (C40)	S A L V E T R T I V R F N R P F L M I I V P T D T Q	
SP11	Rat Serpina3K (C38)	K S L P Q T I P L L N F N R P F M L V I T D D N G Q	
SP12	Human AAT C36	I P P E	V K F N K P F V F L M I E Q N T K
SP13	Human KALLISTATIN (C39)	N R H I L R F N R P F L V V I F S T S T Q	
SP14	Human Antichymotrypsin (C40)	T R T I V R F N R P F L M I I V P T D T Q	
SP15	Rat Serpina3K (C38)	T I P L L N F N R P F M L V I T D D N G Q	
SP16	C36 Core sequence, long		V K F N K P F V F L M I E Q N T K
SP17	C36 Core sequence, short		V K F N K P F V F L M
SP18	Human AAT C36	S I P P E	V K A A A A A F L M I E Q N T K

[0090] SP1 (SEQ ID NO: 18); SP2 (SEQ ID NO: 19); SP3 (SEQ ID NO: 20); SP4 (SEQ ID NO: 21); SP5 (SEQ ID NO: 22); SP6 (SEQ ID NO: 23); SP7 (SEQ ID NO: 24); SP8 (SEQ ID NO: 25); SP9 (SEQ ID NO: 26); SP10 (SEQ ID NO: 27); SP11 (SEQ ID NO: 28); SP12 (SEQ ID NO: 29); SP13 (SEQ ID NO: 30); SP14 (SEQ ID NO: 31); SP15 (SEQ ID NO: 32);

SP16 (SEQ ID NO: 1); SP163M(SEQ ID No: 57) SP17 (SEQ ID NO: 33); SP18 (SEQ ID NO: 34).

[0091] The phrase “consisting essentially of” is herein meant to define the scope of the peptides to the specified material amino acids, and to only include additional amino acids or changes that do not materially affect the claimed invention’s basic and novel characteristics, namely, the anti-inflammatory capacity of the short isolated or synthesized peptides. The definition specifically excludes peptides that have a sequence of a complete Serpin protein, and the definition also specifically excludes peptide sequences that are equal to or longer than 37 amino acids of any naturally occurring Serpin protein.

[0092] Without wishing to be bound by a theory, we have also identified the important amino acids that provide the core, and the possible modifications, for, e.g., anti-inflammatory peptides as manufactured herein. The isolated peptides encompassed by the formulae set forth below are also provided and they can be used to reduce inflammation, treat cytokine storms, enhance cell survival, and/or reduce infarct size.

[0093] Human AAT, antichymotrypsin, and kallistatin have been known to contain elements with anti-inflammatory properties. However, these elements have not been previously identified. We have now established a new family of human Serpin-derived peptides with potent anti-inflammatory effect using, e.g., a mouse endotoxemia model (LPS induced endotoxemia). Based on the efficacy of the peptides in the mouse inflammation model, the peptide size, and the safety profile of the parent protein, the AAT-based peptides, the peptides, such as SP16, and fragments and derivatives thereof provide a novel and improved molecule to treat inflammation in humans.

[0094] Formula I provides a composition comprising a peptide comprising, consisting essentially of or consisting of the amino acid sequence X1-Z1-F-N-R -P-F-X2-Z2-X3-Z3-Q (SEQ ID NO:35) and X1-Z1-F-N-K -P-F-X2-Z2-X3-Z3 (SEQ ID NO: 2) wherein

[0095] X1 is V or L;

[0096] X2 is V, L or M or Nle;

[0097] X3 is M or Nle, I or V;

[0098] Z1 is any amino acid;

[0099] Z2 is a sequence of any two amino acids; and

[00100] Z3 is a sequence any five amino acids, wherein the peptide comprises 37 or fewer amino acids.

[00101] In certain aspects of all the embodiments, the isolated peptide causes a 50% or 75% decrease in serum TNF- α levels compared to the serum level as measured prior to administering the isolated peptide, when administered in an effective amount to a human subject. In some aspects of all the embodiments of the invention, the peptide further comprises a fusion protein. The fusion protein can be selected from an epitope tag and a half-life extender or a combination thereof.

[00102] Formula II provides a composition comprising an isolated peptide comprising, consisting essentially of or consisting of the amino acid sequence X1-Z1-F-N-X2-P-F-X3-Z2-X4-Z3-X5 (SEQ ID NO: 3), wherein

[00103] X1 is V or L;

[00104] X2 is K or R;

[00105] X3 is V, L M, or Nle;

[00106] X4 is M, or Nle, I or V;

[00107] X5 is K or Q;

[00108] Z1 is any amino acid;

[00109] Z2 is a sequence of any two amino acids;

[00110] Z3 is a sequence any five amino acids; and wherein the isolated peptide causes a 75% decrease in serum TNF- α levels compared to the serum levels prior to administering the isolated peptide when administered in an effective amount to a human subject.

[00111] In certain embodiments, the peptide comprising the amino acid sequence of X1-Z1-F-N-X2-P-F-X3-Z2-X4-Z3-X5 (SEQ ID NO: 3) as defined above, includes, at most, 35, 22 or 21 amino acid residues. In certain aspects of all the embodiments of the invention, the peptide further comprises a fusion protein. Specifically the fusion protein can be selected from an epitope tag and a half-life extender or a combination thereof.

[00112] In some aspects of all the methods and uses of the invention, the peptide is SP16.

[00113] In some aspects of all the methods and uses of the invention, the peptide can comprise the peptide of SEQ ID NO: 57. In some aspects of all the methods and uses of the invention, the peptide can consist essentially of the peptide of SEQ ID NO: 57.

[00114] Therefore, the invention also provides methods and uses relating to an isolated peptide consisting of or consisting essentially of the amino acid sequence RFNRPFLR (SEQ ID NO: 4) and RFNKPFLR (SEQ ID NO: 5), which can also be used for the treatment of inflammation. In certain embodiments, the peptide causes a 50% or 75% decrease in serum

TNF- α levels compared to the serum TNF- α levels prior to administering the isolated peptide when administered in an effective amount to a human subject. In some aspects of all the embodiments of the invention, the isolated peptide further comprises a fusion protein. Specifically the fusion protein can be selected from an epitope tag and a half-life extender. In other embodiments, the isolated peptide comprises, at most, 100, 35, 22, 21, 16 or 9 amino acids. In other embodiments, the isolated peptide comprises the amino acid sequence of Z1-RFNRPFLLR-Z2 (SEQ ID NO: 6), and Z1-RFNKPFLR-Z2 (SEQ ID NO: 7) wherein Z1 and Z2 are independently between 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or between 1 and 3, between 1 and 5, between 1 and 6, between 1 and 7, between 1 and 8, between 1 and 9, or between 1 and 10 basic amino acids.

[00115] In some aspects of all the embodiments of the invention, the isolated peptide consists essentially of or consists of the amino acid sequence of RRRFNRPFLLRRR (SEQ ID NO: 8) and RRRFNKPFLRRR (SEQ ID NO: 9). The disclosure also provides a composition comprising a peptide consisting essentially of or consisting of the amino acid sequence of FNRPFLL (SEQ ID NO: 10) and FNKPFL (SEQ ID NO: 11).

[00116] In certain embodiments the isolated peptide comprises 5 or more sequential amino acids from the amino acid sequence of FLMIEQNTK (SEQ ID NO: 36). These peptides can be used to reduce inflammation, enhance cell survival, treat cytokine storms, reduce infarct size and/or reduce TNF- α level in a subject. In certain embodiments, the amount of TNF- α level in the serum is reduced compared to the amount of TNF- α in the serum prior to administering the isolated peptide.

[00117] In some aspects, the method of treatment further comprises analysis of TNF- α serum levels prior to administering the isolated peptide and after administering the isolated peptide. If the TNF- α serum level is decreased less than 30%, the step of administering can be repeated with the same dose or with a larger dose of the peptide compared to the first dose.

[00118] Fragments of any of the peptides described above can vary in size. For example, these fragments can be 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, or 37, amino acids in length.

[00119] The peptides described above can, in addition to treating diseases associated with cytokine storms and/or reducing infarct size, e.g. treatment of AMI, reduce inflammation. The peptides exert anti-inflammatory and immune-modulating effects, and additionally, directly or indirectly stimulate beta cell regeneration. In certain embodiments, these peptides reduce

inflammation by reducing the activity or expression of TNF- α . The activity of TNF- α can be reduced by 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or 100%. The expression of TNF- α can be reduced 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or 100%. When administered therapeutically, the peptide composition typically further comprises a pharmaceutically acceptable solution or carrier.

[00120] The peptides described above can also be used to treat, prevent or improve the symptoms of several pathologies. Accordingly, the disclosure further provides methods of treating a disease associated with a cytokine storm or methods of reducing infarct size, comprising the step of administering any one of the peptides described herein or a combination thereof to a subject in need of treatment. In some aspects, the subject has not been treated with alpha-antitrypsin prior to the treatment. In some aspects, the method comprises a step of assaying whether the individual has increased serum TNF- α levels and if the subject has increased serum TNF- α levels then administering the peptide to the subject, and if not, then not administering the peptide to the subject.

[00121] The disclosure also enables methods of preventing development of a disease associated with a cytokine storm or an preventing an increase in infarct size comprising the step of administering any one of the peptides or a combination thereof to a subject in need of prevention of a disease associated with a cytokine storm or prevention of an increase in infarct size. In some aspects, the subject has not been treated with alpha-antitrypsin prior to the treatment. In some aspects, the method comprises a step of assaying whether the individual has increased serum TNF- α levels and if the subject has increased serum TNF- α levels then administering the peptide to the subject, and if not, then not administering the peptide to the subject. In some aspects of all the embodiments of the invention, the subject in need thereof has not been previously treated with any of the peptides as described herein for any condition.

[00122] In some aspects of all embodiments of the invention the primary purpose of the treatment is to treat or prevent undesired cell death and tissue deterioration associated with deleterious after effects of acute myocardial infarction (AMI); gout; stroke; heart surgery complications; traumatic brain injury. The effective dosage in such applications is measured by determining the effect on prevention or improvement of ischemia reperfusion injury. The dosage is not necessarily the same as in treatment of inflammation alone. Effectiveness can also be measured by determining the level of cell viability and/or cell death, e.g., in a specific organ or tissue.

[00123] For convenience, certain terms employed in the entire application (including the specification, examples, and appended claims) are defined throughout the specification. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

[00124] The term "wild type" refers to the naturally-occurring polynucleotide sequence encoding a protein, or a portion thereof, or protein sequence, or portion thereof, respectively, as it normally exists in vivo.

[00125] The term "mutant" refers to any change in the genetic material of an organism, in particular a change (i.e., deletion, substitution, addition, or alteration) in a wild-type polynucleotide sequence or any change in a wild-type protein sequence. Although it is often assumed that a change in the genetic material results in a change of the function of the protein, the term - "mutant" refers to a change in the sequence of a wild-type protein regardless of whether that change alters the function of the protein (e.g., increases, decreases, imparts a new function), or whether that change has no effect on the function of the protein (e.g., the mutation or variation is silent). The term mutation is used interchangeably herein with polymorphism in this application.

[00126] The terms "polypeptide" and "protein" are used interchangeably to refer to an isolated polymer of amino acid residues, and are not limited to a minimum length unless otherwise defined. Peptides, oligopeptides, dimers, multimers, and the like, are also composed of linearly arranged amino acids linked by peptide bonds, and whether produced biologically and isolated from the natural environment, produced using recombinant technology, or produced synthetically typically using naturally occurring amino acids.

[00127] In some aspects, the polypeptide or protein is a "modified polypeptide" comprising non-naturally occurring amino acids. In some aspects, the polypeptides comprise a combination of naturally occurring and non-naturally occurring amino acids, and in some embodiments, the peptides comprise only non-naturally occurring amino acids.

[00128] In some aspects of all the embodiments of the invention, the peptides or modified peptides further comprise co-translational and post-translational (C-terminal peptide cleavage) modifications, such as, for example, disulfide-bond formation, glycosylation, acetylation, phosphorylation, proteolytic cleavage (e.g., cleavage by furins or metalloproteases), and the like to the extent that such modifications do not affect the anti-inflammatory properties of the isolated peptides or their capacity to improve glycemic control.

[00129] In some aspects of the invention, the polypeptide is altered. The term "altered polypeptide" refers to a peptide that includes alterations, such as deletions, additions, and substitutions (generally conservative in nature as would be known to a person in the art, such as alanines), to the native sequence, as long as the protein maintains the desired activity, i.e., its anti-inflammatory activity or capacity to improve glycemic control or reduce hyperglycemia. These modifications can be deliberate, as through site-directed mutagenesis, or can be accidental, such as through mutations of artificial hosts, such as genetically engineered bacteria, yeast or mammalian cells, that produce the proteins, or errors due to PCR amplification or other recombinant DNA methods. Polypeptides or proteins are composed of linearly arranged amino acids linked by peptide bonds, but in contrast to peptides, have a well-defined conformation. Proteins, as opposed to peptides, generally consist of chains of 50 or more amino acids.

[00130] The term "peptide" as used herein typically refers to a sequence of amino acids made up of a single chain of amino acids joined by peptide bonds. Generally, peptides contain at least two amino acid residues and are less than about 50 amino acids in length, unless otherwise defined.

[00131] "Modified peptide" may include the incorporation of non-natural amino acids into the peptides of the invention, including synthetic non-native amino acids, substituted amino acids, or one or more D-amino acids into the peptides (or other components of the composition, with exception for protease recognition sequences) is desirable in certain situations. D-amino acid-containing peptides exhibit increased stability in vitro or in vivo compared to L-amino acid-containing forms. Thus, the construction of peptides incorporating D-amino acids can be particularly useful when greater in vivo or intracellular stability is desired or required. More specifically, D-peptides are resistant to endogenous peptidases and proteases, thereby providing better oral trans-epithelial and transdermal delivery of linked drugs and conjugates, improved bioavailability of membrane-permanent complexes (see below for further discussion), and prolonged intravascular and interstitial lifetimes when such properties are desirable. The use of D-isomer peptides can also enhance transdermal and oral trans-epithelial delivery of linked drugs and other cargo molecules. Additionally, D-peptides cannot be processed efficiently for major histocompatibility complex class II-restricted presentation to T helper cells, and are therefore less likely to induce humoral immune responses in the whole organism. Peptide conjugates can therefore be constructed using, for example, D-isomer forms of cell penetrating peptide sequences, L-isomer forms of cleavage sites, and D-isomer forms of

therapeutic peptides. Therefore, in some embodiments the peptides as disclosed comprise L and D amino acids, wherein no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 D-amino acids are included. In certain aspects, the peptides comprise more than 10 D-amino acids, and in certain aspects all the amino acids of the peptides are D-amino acids.

[00132] In yet a further aspect, the peptides or fragments or derivatives thereof can be "retro-inverso peptides." A "retro-inverso peptide" refers to a peptide with a reversal of the direction of the peptide bond on at least one position, i.e., a reversal of the amino- and carboxy-termini with respect to the side chain of the amino acid. Thus, a retro-inverso analogue has reversed termini and reversed direction of peptide bonds while approximately maintaining the topology of the side chains as in the native peptide sequence. The retro-inverso peptide can contain L-amino acids or D-amino acids, or a mixture of L-amino acids and D-amino acids, up to all of the amino acids being the D- isomer. Partial retro-inverso peptide analogues are polypeptides in which only part of the sequence is reversed and replaced with enantiomeric amino acid residues. Since the retro-inverted portion of such an analogue has reversed amino and carboxyl termini, the amino acid residues flanking the retro-inverted portion are replaced by side-chain-analogous α -substituted geminal-diaminomethanes and malonates, respectively. Retro-inverso forms of cell penetrating peptides have been found to work as efficiently in translocating across a membrane as the natural forms. Synthesis of retro-inverso peptide analogues are described in Bonelli, F. et al., *Int J Pept Protein Res.* 24(6):553-6 (1984); Verdini, A and Viscomi, G. C, *J. Chem. Soc. Perkin Trans. 1*:697-701 (1985); and U.S. Patent No. 6,261,569, which are incorporated herein in their entirety by reference. Processes for the solid-phase synthesis of partial retro-inverso peptide analogues have been described (EP 97994-B) which is also incorporated herein in its entirety by reference.

[00133] The terms "homology", "identity" and "similarity" refer to the degree of sequence similarity between two peptides or between two optimally aligned nucleic acid molecules. Homology and identity can each be determined by comparing a position in each sequence which can be aligned for purposes of comparison. For example, it is based upon using standard homology software in the default position, such as BLAST, version 2.2.14. When an equivalent position in the compared sequences is occupied by the same base or amino acid, then the molecules are identical at that position; when the equivalent site occupied by similar amino acid residues (e.g., similar in steric and/or electronic nature such as, for example conservative amino acid substitutions), then the molecules can be referred to as homologous (similar) at that

position. Expression as a percentage of homology/similarity or identity refers to a function of the number of similar or identical amino acids at positions shared by the compared sequences, respectfully. A sequence which is "unrelated" or "non-homologous" shares less than 40% identity, though preferably less than 25% identity with the sequences as disclosed herein.

[00134] As used herein, the term "sequence identity" means that two polynucleotide or amino acid sequences are identical (i.e., on a nucleotide-by-nucleotide or residue-by-residue basis) over the comparison window. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, U, or I) or residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the comparison window (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity.

[00135] The term "substantial identity" as used herein denotes a characteristic of a polynucleotide or amino acid sequence, wherein the polynucleotide or amino acid comprises a sequence that has at least 85% sequence identity, preferably at least 90% to 95% sequence identity, more usually at least 99% sequence identity as compared to a reference sequence over a comparison window of at least 18 nucleotide (6 amino acid) positions, frequently over a window of at least 24-48 nucleotide (8-16 amino acid) positions, wherein the percentage of sequence identity is calculated by comparing the reference sequence to the sequence which can include deletions or additions which total 20 percent or less of the reference sequence over the comparison window. The reference sequence can be a subset of a larger sequence. The term "similarity", when used to describe a polypeptide, is determined by comparing the amino acid sequence and the conserved amino acid substitutes of one polypeptide to the sequence of a second polypeptide.

[00136] As used herein, the terms "homologous" or "homologues" are used interchangeably, and when used to describe a polynucleotide or polypeptide, indicates that two polynucleotides or polypeptides, or designated sequences thereof, when optimally aligned and compared, for example using BLAST, version 2.2.14 with default parameters for an alignment (see herein) are identical, with appropriate nucleotide insertions or deletions or amino-acid insertions or deletions, in at least 70% of the nucleotides, usually from about 75% to 99%, and more preferably at least about 98 to 99% of the nucleotides. The term "homolog" or "homologous" as used herein also refers to homology with respect to structure and/or function.

With respect to sequence homology, sequences are homologs if they are at least 50%, at least 60 at least 70%, at least 80%, at least 90%, at least 95% identical, at least 97% identical, or at least 99% identical. Determination of homologs of the genes or peptides of the present invention can be easily ascertained by the skilled artisan.

[00137] The term "substantially homologous" refers to sequences that are at least 90%, at least 95% identical, at least 96%, identical at least 97% identical, at least 98% identical or at least 99% identical. Homologous sequences can be the same functional gene in different species. Determination of homologs of the genes or peptides of the present invention can be easily ascertained by the skilled artisan.

[00138] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[00139] Optimal alignment of sequences for comparison can be conducted, for example, by the local homology algorithm of Smith and Waterman (Adv. Appl. Math. 2:482 (1981), which is incorporated by reference herein), by the homology alignment algorithm of Needleman and Wunsch (J. Mol. Biol. 48:443-53 (1970), which is incorporated by reference herein), by the search for similarity method of Pearson and Lipman (Proc. Natl. Acad. Sci. USA 85:2444-48 (1988), which is incorporated by reference herein), by computerized implementations of these algorithms (e.g., GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection. (See generally Ausubel et al. (eds.), Current Protocols in Molecular Biology, 4th ed., John Wiley and Sons, New York (1999)).

[00140] One example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments to show the percent sequence identity. It also plots a tree or dendrogram showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng and Doolittle (J. Mol. Evol. 25:351-60 (1987), which is incorporated by reference herein). The method used is similar to the method described by Higgins and Sharp (Comput. Appl. Biosci. 5:151-53 (1989), which is incorporated by reference herein). The

program can align up to 300 sequences, each of a maximum length of 5,000 nucleotides or amino acids. The multiple alignment procedure begins with the pairwise alignment of the two most similar sequences, producing a cluster of two aligned sequences. This cluster is then aligned to the next most related sequence or cluster of aligned sequences. Two clusters of sequences are aligned by a simple extension of the pairwise alignment of two individual sequences. The final alignment is achieved by a series of progressive, pairwise alignments. The program is run by designating specific sequences and their amino acid or nucleotide coordinates for regions of sequence comparison and by designating the program parameters. For example, a reference sequence can be compared to other test sequences to determine the percent sequence identity relationship using the following parameters: default gap weight (3.00), default gap length weight (0.10), and weighted end gaps.

[00141] Another example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described by Altschul et al. (J. Mol. Biol. 215:403-410 (1990), which is incorporated by reference herein). (See also Zhang et al., Nucleic Acid Res. 26:3986-90 (1998); Altschul et al., Nucleic Acid Res. 25:3389-402 (1997), which are incorporated by reference herein). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information internet web site. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al. (1990), supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extension of the word hits in each direction is halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff, Proc. Natl. Acad. Sci. USA 89:10915-9 (1992), which is incorporated by reference herein) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

[00142] In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin and Altschul, Proc. Natl. Acad. Sci. USA 90:5873-77 (1993), which is incorporated by reference herein). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, an amino acid sequence is considered similar to a reference amino acid sequence if the smallest sum probability in a comparison of the test amino acid to the reference amino acid is less than about 0.1, more typically less than about 0.01, and most typically less than about 0.001.

[00143] The term "variant" as used herein refers to a peptide or nucleic acid that differs from the polypeptide or nucleic acid by one or more amino acid or nucleic acid deletions, additions, substitutions or side-chain modifications, yet retains one or more specific functions or biological activities of the naturally occurring molecule. Amino acid substitutions include alterations in which an amino acid is replaced with a different naturally-occurring or a non-conventional amino acid residue. Such substitutions may be classified as "conservative", in which case an amino acid residue contained in a polypeptide is replaced with another naturally occurring amino acid of similar character either in relation to polarity, side chain functionality or size. Such conservative substitutions are well known in the art. Substitutions encompassed by the present invention may also be "non-conservative", in which an amino acid residue which is present in a peptide is substituted with an amino acid having different properties, such as naturally-occurring amino acid from a different group (e.g., substituting a charged or hydrophobic amino acid with alanine), or alternatively, in which a naturally-occurring amino acid is substituted with a non-conventional amino acid. In some embodiments amino acid substitutions are conservative. Also encompassed within the term variant when used with reference to a polynucleotide or polypeptide, refers to a polynucleotide or polypeptide that can vary in primary, secondary, or tertiary structure, as compared to a reference polynucleotide or polypeptide, respectively (e.g., as compared to a wild-type polynucleotide or polypeptide).

[00144] Variants can also be synthetic, recombinant, or chemically modified polynucleotides or polypeptides isolated or generated using methods well known in the art. Variants can include conservative or non-conservative amino acid changes, as described below. Polynucleotide changes can result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence. Variants can also include

insertions, deletions or substitutions of amino acids, including insertions and substitutions of amino acids and other molecules) that do not normally occur in the peptide sequence that is the basis of the variant, for example but not limited to insertion of ornithine which do not normally occur in human proteins. The term "conservative substitution," when describing a polypeptide, refers to a change in the amino acid composition of the polypeptide that does not substantially alter the polypeptide's activity. For example, a conservative substitution refers to substituting an amino acid residue for a different amino acid residue that has similar chemical properties.

Conservative amino acid substitutions include replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

[00145] "Conservative amino acid substitutions" result from replacing one amino acid with another having similar structural and/or chemical properties, such as the replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine. Thus, a "conservative substitution" of a particular amino acid sequence refers to substitution of those amino acids that are not critical for polypeptide activity or substitution of amino acids with other amino acids having similar properties (e.g., acidic, basic, positively or negatively charged, polar or non-polar, etc.) such that the substitution of even critical amino acids does not reduce the activity of the peptide, (i.e. the ability of the peptide to penetrate the blood brain barrier (BBB)). Conservative substitution tables providing functionally similar amino acids are well known in the art. For example, the following six groups each contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Serine (S), Threonine (T); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W). (See also Creighton, *Proteins*, W. H. Freeman and Company (1984), incorporated by reference in its entirety.) In some embodiments, individual substitutions, deletions or additions that alter, add or delete a single amino acid or a small percentage of amino acids can also be considered "conservative substitutions" if the change does not reduce the activity of the peptide. Insertions or deletions are typically in the range of about 1 to 5 amino acids. The choice of conservative amino acids may be selected based on the location of the amino acid to be substituted in the peptide, for example if the amino acid is on the exterior of the peptide and expose to solvents, or on the interior and not exposed to solvents.

[00146] In alternative embodiments, one can select the amino acid which will substitute an existing amino acid based on the location of the existing amino acid, i.e. its exposure to

solvents (i.e. if the amino acid is exposed to solvents or is present on the outer surface of the peptide or polypeptide as compared to internally localized amino acids not exposed to solvents). Selection of such conservative amino acid substitutions are well known in the art, for example as disclosed in Dordo et al, J. Mol Biol, 1999, 217, 721-739 and Taylor et al, J. Theor. Biol. 119(1986);205-218 and S. French and B. Robson, J. Mol. Evol. 19(1983)171.

Accordingly, one can select conservative amino acid substitutions suitable for amino acids on the exterior of a protein or peptide (i.e. amino acids exposed to a solvent), for example, but not limited to, the following substitutions can be used: substitution of Y with F, T with S or K, P with A, E with D or Q, N with D or G, R with K, G with N or A, T with S or K, D with N or E, I with L or V, F with Y, S with T or A, R with K, G with N or A, K with R, A with S, K or P.

[00147] In alternative embodiments, one can also select conservative amino acid substitutions encompassed suitable for amino acids on the interior of a protein or peptide, for example one can use suitable conservative substitutions for amino acids is on the interior of a protein or peptide (i.e. the amino acids are not exposed to a solvent), for example but not limited to, one can use the following conservative substitutions: where Y is substituted with F, T with A or S, I with L or V, W with Y, M with L, N with D, G with A, T with A or S, D with N, I with L or V, F with Y or L, S with A or T and A with S, G, T or V. In some embodiments, non-conservative amino acid substitutions are also encompassed within the term of variants.

[00148] The term "derivative" as used herein refers to peptides which have been chemically modified, for example but not limited to by techniques such as ubiquitination, labeling, pegylation (derivatization with polyethylene glycol), lipidation, glycosylation, or addition of other molecules. A molecule also a "derivative" of another molecule when it contains additional chemical moieties not normally a part of the molecule. Such moieties can improve the molecule's solubility, absorption, biological half-life, etc. The moieties can alternatively decrease the toxicity of the molecule, eliminate or attenuate any undesirable side effect of the molecule, etc. Moieties capable of mediating such effects are disclosed in Remington's Pharmaceutical Sciences, 18th edition, A. R. Gennaro, Ed., MackPubl., Easton, PA (1990), incorporated herein, by reference, in its entirety.

[00149] Thus, in certain aspects of all the embodiments of the invention, the peptides of the invention comprise peptide derivatives, such as pegylated peptides.

[00150] The term "functional" when used in conjunction with "derivative" or "variant" refers to a peptide of the invention which possesses a biological activity (either functional or

structural) that is substantially similar to a biological activity of the entity or molecule it is a functional derivative or functional variant thereof, i.e., anti-inflammatory activity in the context of the peptides described herein. The term functional derivative is intended to include the fragments, analogues or chemical derivatives of a molecule.

[00151] The term "insertions" or "deletions" are typically in the range of about 1 to 5 amino acids. The variation allowed can be experimentally determined by producing the peptide synthetically while systematically making insertions, deletions, or substitutions of nucleotides in the sequence using recombinant DNA techniques.

[00152] The term "substitution" when referring to a peptide, refers to a change in an amino acid for a different entity, for example another amino acid or amino-acid moiety. Substitutions can be conservative or non-conservative substitutions.

[00153] An "analog" of a molecule such as a peptide refers to a molecule similar in function to either the entire molecule or to a fragment thereof. The term "analog" is also intended to include allelic species and induced variants. Analogs typically differ from naturally occurring peptides at one or a few positions, often by virtue of conservative substitutions. Analogs typically exhibit at least 80 or 90% sequence identity with natural peptides. Some analogs also include unnatural amino acids or modifications of N or C terminal amino acids. Examples of unnatural amino acids are, for example but not limited to; disubstituted amino acids, N-alkyl amino acids, lactic acid, 4-hydroxyproline, γ -carboxyglutamate, ϵ -N,N,N- trimethyllysine, ϵ -N-acetyllysine, O-phosphoserine, N-acetylserine, N- formylmethionine, 3-methylhistidine, 5-hydroxylysine, σ -N-methylarginine. Fragments and analogs can be screened for prophylactic or therapeutic efficacy in transgenic animal models as described below.

[00154] By "covalently bonded" is meant joined either directly or indirectly (e.g., through a linker) by a covalent chemical bond. In some aspects of all the embodiments of the invention, the fusion peptides are covalently bonded.

[00155] The term "fusion protein" as used herein refers to a recombinant protein of two or more proteins. Fusion proteins can be produced, for example, by a nucleic acid sequence encoding one protein is joined to the nucleic acid encoding another protein such that they constitute a single open-reading frame that can be translated in the cells into a single polypeptide harboring all the intended proteins. The order of arrangement of the proteins can vary. Fusion proteins can include an epitope tag or a half-life extender. Epitope tags include biotin, FLAG tag, c-myc, hemagglutinin, His6 (SEQ ID NO: 37), digoxigenin, FITC, Cy3, Cy5,

green fluorescent protein, V5 epitope tags, GST, β -galactosidase, AU1, AU5, and avidin. Half-life extenders include Fc domain and serum albumin.

[00156] The terms "subject" and "individual" and "patient" are used interchangeably herein, and refer to an animal, for example a human or non-human animal (e.g., a mammal), to whom treatment, including prophylactic treatment, with a pharmaceutical composition as disclosed herein, is provided. The term "subject" as used herein refers to human and non-human animals. The term "non-human animals" includes all vertebrates, e.g., mammals, such as non-human primates, (particularly higher primates), sheep, dogs, rodents (e.g. mouse or rat), guinea pigs, goats, pigs, cats, rabbits, cows, and non-mammals such as chickens, amphibians, reptiles etc. In one embodiment, the subject is human. In another embodiment, the subject is an experimental animal or animal substitute as a disease model. Non-human mammals include mammals such as non-human primates, (particularly higher primates), sheep, dogs, rodents (e.g. mouse or rat), guinea pigs, goats, pigs, cats, rabbits and cows. In some aspects, the non-human animal is a companion animal such as a dog or a cat.

[00157] "Treating" a disease or condition in a subject or "treating" a patient having a disease or condition refers to subjecting the individual to a pharmaceutical treatment, e.g., the administration of a drug, such that at least one symptom of the disease or condition is decreased or stabilized. Typically, when the peptide is administered therapeutically as a treatment, it is administered to a subject who presents with one or more symptoms of, e.g., AMI.

[00158] The term "prevention" is used in connection of prevention of symptoms or slowing down of symptom development from the time of asymptomatic state. Typically, when the peptide is administered preventively, it is administered to a subject who does not present imminent symptoms of a condition described herein, e.g. AMI. Typically, the subject is at risk of developing an infarct or a disease associated with a cytokine storm, such as AMI, due to the family history, laboratory results, genetic testing or life-style. The peptide may also be administered within 10-30 minutes or 10-60 minutes, or within 10 minutes to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours, up to 2, 3, 4, 5, days after onset of symptoms of, e.g., AMI or stroke or after diagnosis of the same to prevent worsening of symptoms. The peptide may also be administered within 10-30 minutes or 10-60 minutes, or within 10 minutes to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours, up to 2, 3, 4, 5, days after injury and/or index event to prevent worsening of symptoms. In some embodiments of any of the aspects described herein, the peptide may be

administered within 10-30 minutes, 30-60 minutes, or 10-60 minutes, after injury and/or index event.

[00159] By "specifically binds" or "specific binding" is meant a compound or antibody that recognizes and binds a desired polypeptide but that does not substantially recognize and bind other molecules in a sample, for example, a biological sample, which naturally includes a polypeptide of the invention. Specific binding can be characterized by a dissociation constant of at least about 1×10^{-6} M or smaller. In other embodiments, the dissociation constant is at least about 1×10^{-7} M, 1×10^{-8} M, or 1×10^{-9} M. Methods for determining whether two molecules specifically bind are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like.

[00160] By "isolated" it is meant that the polypeptide has been separated from any natural environment, such as a body fluid, e.g., blood, and separated from the components that naturally accompany the peptide.

[00161] By isolated and "substantially pure" is meant a polypeptide that has been separated and purified to at least some degree from the components that naturally accompany it. Typically, a polypeptide is substantially pure when it is at least about 60%, or at least about 70%, at least about 80%, at least about 90%, at least about 95%, or even at least about 99%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. For example, a substantially pure polypeptide may be obtained by extraction from a natural source, by expression of a recombinant nucleic acid in a cell that does not normally express that protein, or by chemical synthesis.

[00162] By a "decrease" or "inhibition" used in the context of the level of, for example TNF-alpha levels refers to reduction of the amount of protein in the biological sample, such as blood or tissue sample, a cell, a cell extract, or a cell supernatant. For example, such a decrease may be due to reduced RNA stability, transcription, or translation, increased protein degradation, or RNA interference. Preferably, this decrease is at least about 5%, at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 80%, or even at least about 90% compared to a reference value.

[00163] The term "reference value" in the context of the claims and the application refers typically to an abnormally high TNF-alpha level found in an individual affected with or suffering from inflammation. The reference value is typically the amount of TNF-alpha in the individual prior to administering of the peptide of the invention. In some aspects of all the

embodiments concerning glycemic control, the term "reference value" refers to the numeric values used in measuring glycemic control in a subject. There are a number of tests which can be used to determine if, e.g., a human subject is affected with pre-diabetes. Such tests include, e.g., the A1C test, fasting plasma glucose test (FPG), and the oral glucose tolerance test (OGTT).

[00164] By an "increase" in the expression or activity of a gene or protein is meant a positive change in protein or nucleic acid level or activity in a cell, a cell extract, or a cell supernatant. For example, such an increase may be due to increased RNA stability, transcription, or translation, or decreased protein degradation. Preferably, this increase is at least 5%, at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 80%, at least about 100%, at least about 200%, or even about 500% or more over the level of expression or activity under control conditions.

[00165] The term "recombinant" as used herein to describe a nucleic acid molecule, means a polynucleotide of genomic, cDNA, viral, semisynthetic, and/or synthetic origin, which, by virtue of its origin or manipulation, is not associated with all or a portion of the polynucleotide with which it is associated in nature. The term recombinant as used with respect to a protein or polypeptide, means a polypeptide produced by expression of a recombinant polynucleotide. The term recombinant as used with respect to a host cell means a host cell into which a recombinant polynucleotide has been introduced. Recombinant is also used herein to refer to, with reference to material (e.g., a cell, a nucleic acid, a protein, or a vector) that the material has been modified by the introduction of a heterologous material (e.g., a cell, a nucleic acid, a protein, or a vector).

[00166] The term "vectors" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked; a plasmid is a species of the genus encompassed by "vector". The term "vector" typically refers to a nucleic acid sequence containing an origin of replication and other entities necessary for replication and/or maintenance in a host cell. Vectors capable of directing the expression of genes and/or nucleic acid sequence to which they are operatively linked are referred to herein as "expression vectors". In general, expression vectors of utility are often in the form of "plasmids" which refer to circular double stranded DNA loops which, in their vector form are not bound to the chromosome, and typically comprise entities for stable or transient expression or the encoded DNA. Other expression vectors can be used in the methods as disclosed herein for example, but are not limited to,

plasmids, episomes, bacterial artificial chromosomes, yeast artificial chromosomes, bacteriophages or viral vectors, and such vectors can integrate into the host's genome or replicate autonomously in the particular cell. A vector can be a DNA or RNA vector. Other forms of expression vectors known by those skilled in the art which serve the equivalent functions can also be used, for example self-replicating extrachromosomal vectors or vectors which integrates into a host genome. Preferred vectors are those capable of autonomous replication and/or expression of nucleic acids to which they are linked. Vectors capable of directing the expression of genes to which they are operatively linked are referred to herein as "expression vectors".

[00167] The term "viral vectors" refers to the use of viruses, or virus-associated vectors as carriers of a nucleic acid construct into a cell. Constructs may be integrated and packaged into non-replicating, defective viral genomes like Adenovirus, Adeno-associated virus (AAV), or Herpes simplex virus (HSV) or others, including reteroviral and lentiviral vectors, for infection or transduction into cells. The vector may or may not be incorporated into the cell's genome. The constructs may include viral sequences for transfection, if desired. Alternatively, the construct may be incorporated into vectors capable of episomal replication, e.g. EPV and EBV vectors.

[00168] The articles "a" and "an" are used herein to refer to one or to more than one (i.e., at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element. Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term "about." The term "about" when used in connection with percentages can mean +1%. The present invention is further explained in detail by the following examples, but the scope of the invention should not be limited thereto.

[00169] It should be understood that this invention is not limited to the particular methodology, protocols, and reagents, etc., described herein and as such can vary. The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is defined solely by the claims. Other features and advantages of the invention will be apparent from the following Detailed Description, the drawings, and the claims.

Treatment methods of the invention

[00170] One aspect of the present invention relates to the use of peptides described herein and mutants, variants, analogs or derivatives thereof. Specifically, these methods relate to administering any one of the peptides as described herein or their pharmaceutically acceptable modifications in a pharmaceutically acceptable carrier to a subject, e.g., a mammal in need thereof, e.g., a human, i.e., a subject having a disease associated with a cytokine storm; a subject in need of a reduction of infarct size; and/or a subject in need of treatment of AMI. Clinical descriptions of these diseases and conditions are well known. In some aspects the human is first diagnosed as having one or more symptom of the disease before administering one or more of the peptides of the invention. In some embodiments, the human has not previously been administered AAT as a treatment for the symptoms.

[00171] As used herein, a disease “associated with a cytokine storm” is a disease in which a cytokine storm is a cause of, symptomatic of, or a result of the pathogenesis of the disease. Non-limiting examples of diseases associated with a cytokine storm can include acute myocardial infarction (AMI); gout; stroke; heart surgery complications; and/or traumatic brain injury.

[00172] A subject in need of a reduction in infarct size can be a subject with an infarct. A subject in need of a reduction in infarct size can be a subject having or diagnosed as having, e.g., acute myocardial infarction (AMI); ischemia; stroke; traumatic brain injury; and/or toxic shocks.

[00173] As used herein, “acute myocardial infarction” or “AMI” refers to a reduction or cessation of blood flow to the heart, which can result in damage to the heart tissue from a variety of causes.

[00174] In some embodiments of any of the aspects described herein, the subject does not have a condition, does not have symptoms of the condition, and/or is not diagnosed as having the condition selected from the group consisting of type II diabetes, lupus, graft versus host disease, uveitis, eczema, psoriasis, cystic fibrosis, rheumatoid arthritis, acute radiation syndrome, burn patients, inflammatory bowel disease, type I diabetes, and hyperglycemia.

[00175] For example, we have shown in established preclinical mouse models for human diseases including AMI that, for example, the SP16 peptide significantly improves the symptoms. For example, SP16 was shown to reduce infarct size.

[00176] We have also provided evidence that, e.g., the SP16 peptide can be safely administered using well-established preclinical safety studies. For example, we have also shown that SP16 does not affect heart contractility in the AMI model. Additionally, it has been shown using FastPatch assay that, for example, the SP16 peptide does not impact hERG activity and we also were not able to identify any hits on human receptor panning study (GenSEP Explorer) for the SP16 peptide.

[00177] Therefore, in one aspect we provide methods for treatment of a subject having a disease associated with a cytokine storm; a subject in need of a reduction of infarct size; and/or a subject in need of treatment of AMI, comprising administering to a human subject in need thereof a composition comprising at least one of the peptides of the invention. In some aspects, the peptide comprises SP16.

[00178] We have shown that the peptides of the invention, e.g., SP16 are toll like receptor-2 agonists. Accordingly, without wishing to be bound by a theory, the peptides, such as SP16, act as an anti-inflammatory drug by promoting an anti-inflammatory cytokine profile. Also, without wishing to be bound by a theory, the peptides, such as SP16, also can act as immune modulators by inducing expansion of tolerogenic and protective T-regulatory cells (T-regs). Further, without wishing to be bound by a theory, the peptides, such as SP16, also can down-regulate autoimmune responses without inducing general immunological suppression thereby providing a superior treatment for autoimmune diseases compared to most of the currently available treatments which generally suppress the immune system exposing the treated individuals to a risk of infections while treated with the general immunosuppressants.

[00179] As the peptides are derived from AAT, and in view of our results in vivo and in vitro models, it is reasonable to expect most of the AAT's therapeutic effects to apply also to the peptides of the invention, such as SP16. Specifically, AAT has been shown to modulate T-cell proliferation and NF-kappa-B activation; impair NK target cell interaction; inhibit serine proteases activation of epithelial cell EGFR/TLR-4 signaling; be involved in TNF-alpha-induced gene expression and apoptosis of endothelial cells; prevent red blood cell haemolysis by *E. coli*, decrease circulating eosinophil cell count; inhibit neutrophil chemotaxis, NADHP oxidase and ANCA signaling; inhibit monocyte and macrophage cytokine release and regulation of CD14 expression, and inhibit mast cell histamine release; and modulate B-cell proliferation and cytokine production. In some aspects, the peptide is SP16, which may

comprise one or more modifications typically performed to enhance peptide bioavailability and/or shelf life, such as pegylation and the like.

[00180] We also performed a peptide optimization assay using an alanine scan with the TLR-2 assay. Data was obtained using an experiment with an engineered TLR-2 indicator cell line (HEK-BLUE™ mTLR2, Invivogen). Cells were incubated with the 20 µg/ml of the indicated peptides for 24 hours. Upon TLR2 activation, the cells secrete alkaline phosphatase, which can be assayed. The assay was done in triplicate and averages are plotted. Peptide SP34 is a scrambled peptide control (Yellow), and PAM (Pam3CSK4; Red) is a positive control. See, e.g., PCT/US13/20498; which is incorporated by reference herein in its entirety.

[00181] The following table provides the results from an assay for SP16's pharmacokinetic profile.

PK parameters	SP16, IV (5 mg/kg)
C0 (µg/mL)	2.5
AUC to Last (µg-hr/mL)	0.9
t1/2 (hr)	1.9
Total CL (mL/hr)	140
Total CL (mL/min/kg)	9.7
Last Time point	8.0
MRTINF (hr)	1.1
V (mL)	374
Vss (mL)	149

[00182] In performing the assay, three normal rats were injected intravenously with 5 mg/kg SP16 and the plasma concentration of SP16 established at 8 time points following the injection. For each timepoint, SP16 levels were determined by LC/MS/MS and the values used to calculate the Cmax (2.5 ug/ml) and T1/2 (1.9 hours). The assay was executed by Apredica, Boston, MA. Accordingly, we determined that SP16 has a half-life of 1.9 hours in normal rats.

[00183] The SP16 safety profile also included hERG data. The hERG FastPatch assay showed that SP16 does not inhibit hERG at up to 25 µM doses. This data predicts that SP16 will not have cardiac safety issues in humans. The study was executed by Apredica, Boston, MA.

[00184]

Client ID	Test conc (μ M)	IC50 value (μ M)	comment
SP16	0.008-25	>25	No concentration-dependent inhibition observed.
Quinidine	0.01-10	1.8	positive control

Customer Id	Mean % Activity							Comments
	0 μ M	0.008 μ M	0.04 μ M	0.2 μ M	1 μ M	5 μ M	25 μ M	
SP16	100	89	95	95	78	88	89	No concentration-dependent inhibition observed.

[00185] In addition, we also performed a profile using human receptor panning. The GenSEP Explorer panel contains 111 in vitro assay targets carefully selected to assess drug/chemical biological activities. Assay categories include GPCRs, Voltage-Gated Ion Channels, Ligand-Gated Ion Channels, Neurotransmitter transporters, Nuclear Receptors and Steroids as well as a diverse set of biochemical targets including phosphodiesterases, kinases and other relevant enzymes. The study was executed by Caliper LifeSciences, and the results are summarized in the table below. It appears that SP16 has no effect on 111 human receptors, indicating that SP16 has an excellent human safety profile.

Receptor Class	Number of receptors tested	Percent inhibition
Neurotransmitter Related	47	Insignificant
Steroids	4	Insignificant
Ion Channels	8	Insignificant
Growth factors & hormones	2	Insignificant
Second Messengers	3	Insignificant
Brain/gut peptides	15	Insignificant

Enzymes	20	Insignificant
Enzymes, Kinases	11	Insignificant
Cell-Based, Functional	1	Insignificant

[00186] In view of the safety profile of SP16, we can reasonably extrapolate that the other peptide fragments provided herein would also be safe for administering to human.

[00187] In one embodiment, the methods of treatment described herein, further comprise selection or diagnosis of a subject having any of the above-described conditions, e.g., one arising from inflammation prior to administering a peptide as disclosed herein or a mutant, variant, analog or derivative thereof, to thereby treat the condition or dysfunction, such as inflammation. Such selection is performed by the skilled practitioner by a number of available methods, for instance, assessment of symptoms which are described herein. For example, AMI can be diagnosed by means of, e.g., an electrocardiogram, a chest radiograph, or the level of troponins. For example, one can assess the amount of TNF-alpha in the subject to determine the amount of inflammation present in the subject.

[00188] In some aspects of all the embodiments, one may use C-reactive protein as a marker for inflammation or treatment efficacy. C-reactive protein (CRP) is used to detect inflammation if there is a high suspicion of tissue injury or infection somewhere in the body. CRP serves as a general marker for infection and inflammation and can be used to evaluate an individual for an acute or chronic inflammatory condition. A high or increasing amount of CRP in the blood suggests the presence of inflammation. In individuals suspected of having a serious bacterial infection, a high CRP suggests the presence of one. In people with chronic inflammatory conditions, high levels of CRP suggest a flare-up or that treatment has not been effective. Normal concentration in healthy human serum is usually lower than 10 mg/L, slightly increasing with aging. Higher levels are found in late pregnant women, mild inflammation and viral infections (10-40 mg/L), active inflammation, bacterial infection (40–200 mg/L), severe bacterial infections and burns (>200 mg/L). In some aspects the term “reference value” refers to the measurements of CRP when CRP is used as a diagnostic test for inflammation.

[00189] Successful or effective treatment is evidenced by amelioration of one or more symptoms of the condition or dysfunction as discussed herein. Administering a peptide as disclosed herein or a mutant, variant, analog or derivative thereof in a subject in need thereof is expected to prevent or retard the development of the conditions and physical dysfunctions described herein (e.g., those arising from an infarct, a cytokine storm, inflammation or auto-immune tissue destruction or to a condition ameliorated by stimulation of expansion of beta cell mass in an individual with diabetes). The term "prevention" is used to refer to a situation wherein a subject does not yet have the specific condition being prevented, meaning that it has not manifested in any appreciable form. Prevention encompasses prevention or slowing of onset and/or severity of a symptom, (including where the subject already has one or more symptoms of another condition). Prevention is performed generally in a subject who is at risk for development of a condition or physical dysfunction. Such subjects are said to be in need of prevention. For example, reduction in the TNF-alpha levels compared to the levels prior to administering the peptides of the invention, would be evidence of successful treatment.

[00190] In one embodiment, the methods of prevention described herein, further comprise selection of such a subject at risk for a condition, e.g., those arising from an infarct, a cytokine storm, inflammation or auto-immune tissue destruction or physical dysfunction as described herein, prior to administering a peptide or a mutant, variant, analog or derivative thereof, in the subject, to thereby prevent the condition or dysfunction. Such selection is performed by the skilled practitioner by a number of available methods. For instance, assessment of risk factors or diagnosis of a disease which is known to cause the condition or dysfunction, or treatment or therapy known to cause the condition or dysfunction. Subjects which have a disease or injury or a relevant family history which is known to contribute to the condition are generally considered to be at increased risk.

[00191] As used herein, the terms "treat" or "treatment" or "treating" refers to therapeutic treatment measures, wherein the object is to prevent or slow the development of the disease, such as reducing at least one effect or symptom of a condition, disease or disorder associated with inflammation. Treatment is generally "effective" if one or more symptoms are improved or clinical markers, such as troponins, TNF-alpha, CRP, blood glucose and/or HbA1c, levels are within normal values or closer to the normal reference values than abnormal values reflecting inflammation or poor glycemic control, depending on the condition, as that term is defined herein. Alternatively, treatment is "effective" if the progression of a disease is slowed down,

exhibition of a symptom or a marker for a disease is reduced. That is, "treatment" includes the improvement of symptoms or markers, slowing of progress or slowing of worsening of at least one symptom that would be expected in absence of treatment. Beneficial or desired clinical results include, but are not limited to, alleviation of one or more symptom(s), diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include patients with one or more symptoms of an infarct, cytokine storm, or inflammation, such as symptoms associated with AMI.

[00192] TNF-alpha levels can be assessed, for example, using any number of readily available commercial ELISA kits.

[00193] In some aspects, the invention relates to methods of preventing a cytokine storm, an infarct, and/or AMI by administering the peptides as described to an individual not yet presenting symptoms of a cytokine storm, an infarct, and/or AMI. For example, the peptides can be administered to an individual at high risk of developing AMI, but not yet having AMI to assist in slowing down the development or preventing the development of AMI.

[00194] The term "effective amount" as used herein refers to the amount of a pharmaceutical composition comprising one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof, to decrease at least one or more symptom of the disease or disorder, and relates to a sufficient amount of pharmacological composition to provide the desired effect. The phrase "therapeutically effective amount" as used herein means a sufficient amount of the composition to treat a disorder, at a reasonable benefit/risk ratio applicable to any medical treatment. The term "therapeutically effective amount" therefore refers to an amount of the composition as disclosed herein that is sufficient to effect a therapeutically or prophylactically reduction in a symptom or clinical marker associated with increased levels of inflammation, infarct, or cytokine storm when administered to a typical subject who has, e.g., AMI. Typically reduction of more than 20% of a disease marker, such as an inflammatory marker, e.g., TNF-alpha, is indicative of effective treatment. In some instances, reduction of more than 50% or more than 75% from the amount of TNF-alpha levels in the individual prior to administering the peptides of the invention is indicative of effective treatment.

[00195] A therapeutically or prophylactically significant reduction in a symptom is, e.g. at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%,

at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 125%, at least about 150% or more in a measured parameter as compared to a control or non-treated subject or the state of the subject prior to administering the peptide. Measured or measurable parameters include clinically detectable markers of disease, for example, elevated or depressed levels of a biological marker, such as TNF- α , as well as parameters related to a clinically accepted scale of symptoms or markers for infarct, cytokine storms, and inflammation. It will be understood, however, that the total daily usage of the compositions and formulations as disclosed herein will be decided by the attending physician within the scope of sound medical judgment. The exact amount required will vary depending on factors such as the type of disease being treated, gender, age, and weight of the subject.

[00196] With reference to the treatment a subject having a disease associated with a cytokine storm; a subject in need of a reduction of infarct size; and/or a subject in need of treatment of AMI, the term "therapeutically effective amount" refers to the amount that is safe and sufficient to delay the development of one or more symptom and results in decrease in the amount of a disease marker, e.g., TNF- α or CRP concentrations. The effective amount for the treatment of a disease depends on the type of disease, the species being treated, the age and general condition of the subject, the mode of administration and so forth. Thus, it is not possible to specify the exact "effective amount." However, for any given case, an appropriate "effective amount" can be determined by one of ordinary skill in the art using only routine experimentation. The efficacy of treatment can be judged by an ordinarily skilled practitioner, for example, efficacy can be assessed in known animal models of inflammation (e.g. LPS model), AMI (e.g. an isoproterenol model), auto-immune tissue destruction (e.g. CAIA model) or diabetes (e.g. db/db mouse model). When using an experimental animal model, efficacy of treatment is evidenced when a reduction in a symptom of a cytokine storm or infarct is shown versus untreated animals.

[00197] In some embodiments of any of the aspects described herein, the subject does not have, does not have symptoms of, or is not diagnosed as having a condition selected from the group consisting of type II diabetes, lupus, graft versus host disease, uveitis, eczema, psoriasis, cystic fibrosis, rheumatoid arthritis, acute radiation syndrome, burn patients, inflammatory bowel disease, type I diabetes, and hyperglycemia. In some embodiments of any of the aspects described herein, the subject does not have increased TNF- α levels. In some embodiments of any of the aspects described herein, the subject is not in need of a reduction in TNF- α levels.

[00198] As used herein, the terms "administering," and "introducing" are used interchangeably herein and refer to the placement of the therapeutic agents such as one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof into a subject by a method or route which results in delivering of such agent(s) at a desired site. The compounds can be administered by any appropriate route which results in an effective treatment in the subject.

[00199] The one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof may be administered by any route known in the art or described herein, for example, oral, parenteral (e.g., intravenously or intramuscularly), intraperitoneal, rectal, cutaneous, nasal, vaginal, inhalant, skin (patch), or ocular. The one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof may be administered in any dose or dosing regimen. One can also use pumps, like the ones used for insulin administration. In some embodiments, the one or more peptides can be administered orally. As depicted in Figure 16 of US Patent 8,975,224; which is incorporated by reference herein in its entirety), oral administration of SP16 was demonstrated to be efficacious, e.g. compared to intraperitoneal injection of SP16 and dexamethasone.

Dosage

[00200] With respect to the therapeutic methods of the invention, it is not intended that the administration of the one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof and be limited to a particular mode of administration, dosage, or frequency of dosing; the present invention contemplates all modes of administration, including intramuscular, intravenous, intraperitoneal, intravesicular, intraarticular, intralesional, subcutaneous, or any other route sufficient to provide a dose adequate to treat the, e.g., inflammation, infarct, or cytokine storm-related disorder. The therapeutic may be administered to the patient in a single dose or in multiple doses. When multiple doses are administered, the doses may be separated from one another by, for example, one hour, three hours, six hours, eight hours, one day, two days, one week, two weeks, or one month. For example, the therapeutic may be administered for, e.g., 2, 3, 4, 5, 6, 7, 8, 10, 15, 20, or more weeks. It is to be understood that, for any particular subject, specific dosage regimes should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions. For example, the dosage of

the therapeutic can be increased if the lower dose does not provide sufficient therapeutic activity.

[00201] While the attending physician ultimately will decide the appropriate amount and dosage regimen, therapeutically effective amounts of the one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof may be provided at a dose of 0.0001, 0.01, 0.01 0.1, 1, 5, 10, 25, 50, 100, 500, or 1,000 mg/kg or µg/kg. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test bioassays or systems.

[00202] Dosages for a particular patient or subject can be determined by one of ordinary skill in the art using conventional considerations, (e.g. by means of an appropriate, conventional pharmacological protocol). A physician may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. The dose administered to a patient is sufficient to effect a beneficial therapeutic response in the patient over time, or, e.g., to reduce symptoms, or other appropriate activity, depending on the application. The dose is determined by the efficacy of the particular formulation, and the activity, stability or serum half-life of the one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof and the condition of the patient, as well as the body weight or surface area of the patient to be treated. The size of the dose is also determined by the existence, nature, and extent of any adverse side-effects that accompany the administration of a particular vector, formulation, or the like in a particular subject. Therapeutic compositions comprising one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof are optionally tested in one or more appropriate in vitro and/or in vivo animal models of disease, such as models of inflammation or diabetes, to confirm efficacy, tissue metabolism, and to estimate dosages, according to methods well known in the art. In particular, dosages can be initially determined by activity, stability or other suitable measures of treatment vs. non-treatment (e.g., comparison of treated vs. untreated cells or animal models), in a relevant assay. Formulations are administered at a rate determined by the LD50 of the relevant formulation, and/or observation of any side-effects of one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof. Administration can be accomplished via single or divided doses.

[00203] In determining the effective amount of one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof to be administered in the treatment or

prophylaxis of disease the physician evaluates circulating plasma levels, formulation toxicities, and progression of the disease.

[00204] The efficacy and toxicity of the compound can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose is effective in 50% of the population) and LD50 (the dose is lethal to 50% of the population). The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. Pharmaceutical compositions which exhibit large therapeutic indices are preferred.

[00205] These compounds may be administered to humans and other animals for therapy by any suitable route of administration that works for small peptides, including orally, nasally, as by, for example, a spray, rectally, intravaginally, parenterally, intracisternally and topically, as by powders, ointments or drops, including buccally and sublingually.

[00206] Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular subject, composition, and mode of administration, without being toxic to the subject.

[00207] The selected dosage level will depend upon a variety of factors including the activity of the particular compound of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

Formulation of pharmaceutical compositions – “pharmaceutically acceptable carriers”

[00208] The administration of one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof may be by any suitable means that results in a concentration of the protein that treats the disorder. The compound may be contained in any appropriate amount in any suitable carrier substance, and is generally present in an amount of 1-95% by weight of the total weight of the composition. The composition may be provided in a dosage form that is suitable for the oral, parenteral (e.g., intravenously or intramuscularly), intraperitoneal, rectal, cutaneous, nasal, vaginal, inhalant, skin (patch), or ocular administration route. Thus, the

composition may be in the form of, e.g., tablets, capsules, pills, powders, granulates, suspensions, emulsions, solutions, gels including hydrogels, pastes, ointments, creams, plasters, drenches, osmotic delivery devices, suppositories, enemas, injectables, implants, sprays, or aerosols. The pharmaceutical compositions may be formulated according to conventional pharmaceutical practice (see, e.g., Remington: The Science and Practice of Pharmacy, 20th edition, 2000, ed. A.R. Gennaro, Lippincott Williams & Wilkins, Philadelphia, and Encyclopedia of Pharmaceutical Technology, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York, incorporated, herein, by reference in its entirety).

[00209] Pharmaceutical compositions according to the invention may be formulated to release the active compound immediately upon administration or at any predetermined time or time period after administration. The latter types of compositions are generally known as controlled release formulations, which include (i) formulations that create substantially constant concentrations of the agent(s) of the invention within the body over an extended period of time; (ii) formulations that after a predetermined lag time create substantially constant concentrations of the agent(s) of the invention within the body over an extended period of time; (iii) formulations that sustain the agent(s) action during a predetermined time period by maintaining a relatively constant, effective level of the agent(s) in the body with concomitant minimization of undesirable side effects associated with fluctuations in the plasma level of the agent(s) (sawtooth kinetic pattern); (iv) formulations that localize action of agent(s), e.g., spatial placement of a controlled release composition adjacent to or in the diseased tissue or organ; (v) formulations that achieve convenience of dosing, e.g., administering the composition once per week or once every two weeks; and (vi) formulations that target the action of the agent(s) by using carriers or chemical derivatives to deliver the therapeutic to a particular target cell type. Administration of the protein in the form of a controlled release formulation is especially preferred for compounds having a narrow absorption window in the gastrointestinal tract or a relatively short biological half-life.

[00210] Any of a number of strategies can be pursued in order to obtain controlled release in which the rate of release outweighs the rate of metabolism of the compound in question. In one example, controlled release is obtained by appropriate selection of various formulation parameters and ingredients, including, e.g., various types of controlled release compositions and coatings. Thus, the protein is formulated with appropriate excipients into a pharmaceutical composition that, upon administration, releases the protein in a controlled manner. Examples

include single or multiple unit tablet or capsule compositions, oil solutions, suspensions, emulsions, microcapsules, molecular complexes, microspheres, nanoparticles, patches, and liposomes.

[00211] As used herein, the phrases "parenteral administration" and "administered parenterally" as used herein mean modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intraventricular, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, sub capsular, subarachnoid, intraspinal, intracerebrospinal, and intrasternal injection and infusion. The phrases "systemic administration," "administered systemically", "peripheral administration" and "administered peripherally" as used herein mean the administration therapeutic compositions other than directly into a tumor such that it enters the animal's system and, thus, is subject to metabolism and other like processes.

[00212] The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. The phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in maintaining the activity of or carrying or transporting the subject agents from one organ, or portion of the body, to another organ, or portion of the body. In addition to being "pharmaceutically acceptable" as that term is defined herein, each carrier must also be "acceptable" in the sense of being compatible with the other ingredients of the formulation. The pharmaceutical formulation comprising the one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof in combination with one or more pharmaceutically acceptable ingredients. The carrier can be in the form of a solid, semi-solid or liquid diluent, cream or a capsule. These pharmaceutical preparations are a further object of the invention. Usually the amount of active compounds is between 0.1-95% by weight of the preparation, preferably between 0.2-20% by weight in preparations for parenteral use and preferably between 1 and 50% by weight in preparations for oral administration. For the clinical use of the methods of the present invention, targeted delivery composition of the invention is formulated

into pharmaceutical compositions or pharmaceutical formulations for parenteral administration, e.g., intravenous; mucosal, e.g., intranasal; enteral, e.g., oral; topical, e.g., transdermal; ocular, e.g., via corneal scarification or other mode of administration. The pharmaceutical composition contains a compound of the invention in combination with one or more pharmaceutically acceptable ingredients. The carrier can be in the form of a solid, semi-solid or liquid diluent, cream or a capsule.

[00213] The term "pharmaceutically acceptable carriers" is intended to include all solvents, diluents, or other liquid vehicle, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Typically, such compounds are carried or transported from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its functional derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations.

[00214] The term "pharmaceutical composition" is used herein refer to compositions or formulations that usually comprise an excipient, such as a pharmaceutically acceptable carrier that is conventional in the art and that is suitable for administration to mammals, and preferably humans or human cells. Such compositions can be specifically formulated for administration via one or more of a number of routes, including but not limited to, oral, ocular, parenteral, intravenous, intraarterial, subcutaneous, intranasal, sublingual, intraspinal, intracerebroventricular, and the like. In addition, compositions for topical (e.g., oral mucosa, respiratory mucosa) and/or oral administration can form solutions, suspensions, tablets, pills, capsules, sustained-release formulations, oral rinses, or powders, as known in the art are

described herein. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, University of the Sciences in Philadelphia (2005)

Remington: The Science and Practice of Pharmacy with Facts and Comparisons, 21st Ed.

[00215] In certain embodiments, the compounds of the present invention may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable bases. The term "pharmaceutically acceptable salts, esters, amides, and prodrugs" as used herein refers to those carboxylate salts, amino acid addition salts, esters, amides, and prodrugs of the compounds of the present invention which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of patients without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio, and effective for their intended use of the compounds of the invention. The term "salts" refers to the relatively non-toxic, inorganic and organic acid addition salts of compounds of the present invention. These salts can be prepared in situ during the final isolation and purification of the compounds or by separately reacting the purified compound in its free base form with a suitable organic or inorganic acid and isolating the salt thus formed. These may include cations based on the alkali and alkaline earth metals such as sodium, lithium, potassium, calcium, magnesium and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations including, but not limited to ammonium, tetramethylanunonium, tetraethyl ammonium, methyl amine, dimethyl amine, trimethylamine, triethylamine, ethylamine, and the like (see, e.g., Berge S. M., et al. (1977) J. Pharm. Sci. 66, 1, which is incorporated herein by reference).

[00216] The term "pharmaceutically acceptable esters" refers to the relatively nontoxic, esterified products of the compounds of the present invention. These esters can be prepared in situ during the final isolation and purification of the compounds, or by separately reacting the purified compound in its free acid form or hydroxyl with a suitable esterifying agent. Carboxylic acids can be converted into esters via treatment with an alcohol in the presence of a catalyst. The term is further intended to include lower hydrocarbon groups capable of being solvated under physiological conditions, e.g., alkyl esters, methyl, ethyl and propyl esters.

[00217] As used herein, "pharmaceutically acceptable salts or prodrugs" are salts or prodrugs that are, within the scope of sound medical judgment, suitable for use in contact with the tissues of subject without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio, and effective for their intended use.

[00218] The term "prodrug" refers to compounds that are rapidly transformed in vivo to yield the functionally active one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof. A thorough discussion is provided in T. Higachi and V. Stella, "Pro-drugs as Novel Delivery Systems," Vol. 14 of the A. C. S. Symposium Series, and in Bioreversible Carriers in: Drug Design, ed. Edward B. Roche, American Pharmaceutical Association and Pergamon Press, 1987, both of which are hereby incorporated by reference. As used herein, a prodrug is a compound that, upon in vivo administration, is metabolized or otherwise converted to the biologically, pharmaceutically or therapeutically active form of the compound. A prodrug of the one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof can be designed to alter the metabolic stability or the transport characteristics of one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof, to mask side effects or toxicity, to improve the flavor of a compound or to alter other characteristics or properties of a compound. By virtue of knowledge of pharmacodynamic processes and drug metabolism in vivo, once a pharmaceutically active form of the one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof, those of skill in the pharmaceutical art generally can design prodrugs of the compound (see, e.g., Nogrady (1985) Medicinal Chemistry A Biochemical Approach, Oxford University Press, N. Y., pages 388-392). Conventional procedures for the selection and preparation of suitable prodrugs are described, for example, in "Design of Prodrugs," ed. H. Bundgaard, Elsevier, 1985. Suitable examples of prodrugs include methyl, ethyl and glycerol esters of the corresponding acid.

Parenteral compositions

[00219] The pharmaceutical composition may be administered parenterally by injection, infusion, or implantation (subcutaneous, intravenous, intramuscular, intraperitoneal, or the like) in dosage forms, formulations, or via suitable delivery devices or implants containing conventional, non-toxic pharmaceutically acceptable carriers and adjuvants. The formulation and preparation of such compositions are well known to those skilled in the art of pharmaceutical formulation.

[00220] Compositions for parenteral use may be provided in unit dosage forms (e.g., in single-dose ampoules), or in vials containing several doses and in which a suitable preservative may be added (see below). The composition may be in form of a solution, a suspension, an emulsion, an infusion device, or a delivery device for implantation, or it may be presented as a

dry powder to be reconstituted with water or another suitable vehicle before use. Apart from the active agent(s), the composition may include suitable parenterally acceptable carriers and/or excipients. The active agent(s) may be incorporated into microspheres, microcapsules, nanoparticles, liposomes, or the like for controlled release. Furthermore, the composition may include suspending, solubilizing, stabilizing, pH-adjusting agents, tonicity adjusting agents, and/or dispersing agents.

[00221] As indicated above, the pharmaceutical compositions according to the invention may be in a form suitable for sterile injection. To prepare such a composition, the suitable active agent(s) are dissolved or suspended in a parenterally acceptable liquid vehicle. Among acceptable vehicles and solvents that may be employed are water, water adjusted to a suitable pH by addition of an appropriate amount of hydrochloric acid, sodium hydroxide or a suitable buffer, 1,3-butanediol, Ringer's solution, dextrose solution, and isotonic sodium chloride solution. The aqueous formulation may also contain one or more preservatives (e.g., methyl, ethyl or n-propyl p-hydroxybenzoate). In cases where one of the compounds is only sparingly or slightly soluble in water, a dissolution enhancing or solubilizing agent can be added, or the solvent may include 10- 60% w/w of propylene glycol or the like.

[00222] Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

[00223] Examples of pharmaceutically acceptable antioxidants include: water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfate, sodium sulfite and the like; oil- soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

[00224] Formulations of the present invention include those suitable for intravenous, oral, nasal, topical, transdermal, buccal, sublingual, rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect.

[00225] Methods of preparing these formulations or compositions include the step of bringing into association a compound of the present invention with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a compound of the present invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

[00226] Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a compound of the present invention as an active ingredient. A compound of the present invention may also be administered as a bolus, electuary or paste.

[00227] Pharmaceutical compositions of this invention suitable for parenteral administration comprise one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

[00228] Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions comprising one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

[00229] These compositions can also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include

isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

[00230] In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

[00231] Injectable depot forms are made by forming microencapsulated matrices of the subject compounds in biodegradable polymers such as polylactide- polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug, such as one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof in liposomes or microemulsions which are compatible with body tissue.

[00232] Regardless of the route of administration selected, the compounds of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of ordinary skill in the art.

Controlled release parenteral compositions

[00233] Controlled release parenteral compositions may be in form of aqueous suspensions, microspheres, microcapsules, magnetic microspheres, oil solutions, oil suspensions, or emulsions. The composition may also be incorporated in biocompatible carriers, liposomes, nanoparticles, implants, or infusion devices.

[00234] Materials for use in the preparation of microspheres and/or microcapsules are, e.g., biodegradable/bioerodible polymers such as polygalactia poly-(isobutyl cyanoacrylate), poly(2-hydroxyethyl-L-glutamine), poly(lactic acid), polyglycolic acid, and mixtures thereof. Biocompatible carriers that may be used when formulating a controlled release parenteral formulation are carbohydrates (e.g., dextrans), proteins (e.g., albumin), lipoproteins, or

antibodies. Materials for use in implants can be nonbiodegradable (e.g., polydimethyl siloxane) or biodegradable (e.g., poly(caprolactone), poly(lactic acid), poly(glycolic acid) or poly(ortho esters)) or combinations thereof.

Solid dosage forms for oral use

[00235] Formulations for oral use include tablets containing the active ingredient(s) in a mixture with non-toxic pharmaceutically acceptable excipients, and such formulations are known to the skilled artisan (e.g., U.S. Patent Nos.: 5,817,307; 5,824,300; 5,830,456; 5,846,526; 5,882,640; 5,910,304; 6,036,949; 6,036,949; and 6,372,218 hereby incorporated by reference). These excipients may be, for example, inert diluents or fillers (e.g., sucrose, sorbitol, sugar, mannitol, microcrystalline cellulose, starches including potato starch, calcium carbonate, sodium chloride, lactose, calcium phosphate, calcium sulfate, or sodium phosphate); granulating and disintegrating agents (e.g., cellulose derivatives including microcrystalline cellulose, starches including potato starch, croscarmellose sodium, alginates, or alginic acid); binding agents (e.g., sucrose, glucose, sorbitol, acacia, alginic acid, sodium alginate, gelatin, starch, pregelatinized starch, microcrystalline cellulose, magnesium aluminum silicate, carboxymethylcellulose sodium, methylcellulose, hydroxypropyl methylcellulose, ethylcellulose, polyvinylpyrrolidone, or polyethylene glycol); and lubricating agents, glidants, and anti-adhesives (e.g., magnesium stearate, zinc stearate, stearic acid, silicas, hydrogenated vegetable oils, or talc). Other pharmaceutically acceptable excipients can be colorants, flavoring agents, plasticizers, humectants, buffering agents, and the like.

[00236] The tablets may be uncoated or they may be coated by known techniques, optionally to delay disintegration and absorption in the gastrointestinal tract and thereby providing a sustained action over a longer period. The coating may be adapted to release the protein in a predetermined pattern (e.g., in order to achieve a controlled release formulation) or it may be adapted not to release the agent(s) until after passage of the stomach (enteric coating). The coating may be a sugar coating, a film coating (e.g., based on hydroxypropyl methylcellulose, methylcellulose, methyl hydroxyethylcellulose, hydroxypropylcellulose, carboxymethylcellulose, acrylate copolymers, polyethylene glycols and/or polyvinylpyrrolidone), or an enteric coating (e.g., based on methacrylic acid copolymer, cellulose acetate phthalate, hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate succinate, polyvinyl acetate phthalate, shellac, and/or ethylcellulose).

Furthermore, a time delay material such as, e.g., glyceryl monostearate or glyceryl distearate, may be employed.

[00237] The solid tablet compositions may include a coating adapted to protect the composition from unwanted chemical changes, (e.g., chemical degradation prior to the release of the active substances). The coating may be applied on the solid dosage form in a similar manner as that described in Encyclopedia of Pharmaceutical Technology, *supra*.

[00238] The compositions of the invention may be mixed together in the tablet, or may be partitioned. In one example, a first agent is contained on the inside of the tablet, and a second agent is on the outside, such that a substantial portion of the second agent is released prior to the release of the first agent.

[00239] Formulations for oral use may also be presented as chewable tablets, or as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent (e.g., potato starch, lactose, microcrystalline cellulose, calcium carbonate, calcium phosphate, or kaolin), or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin, or olive oil. Powders and granulates may be prepared using the ingredients mentioned above under tablets and capsules in a conventional manner using, e.g., a mixer, a fluid bed apparatus, or spray drying equipment.

[00240] In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; humectants, such as glycerol; disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; solution retarding agents, such as paraffin; absorption accelerators, such as quaternary ammonium compounds; wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; absorbents, such as kaolin and bentonite clay; lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin

capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

[00241] A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

[00242] The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients. In one aspect, a solution of resolvins and/or protectins or precursor or analog thereof can be administered as eye drops for ocular neovascularization or ear drops to treat otitis.

[00243] Oral administration of the peptides useful according to this invention have been shown to work as discussed, e.g., in U.S. Patent No. 8,975,224.

[00244] Oral administration of peptides has been shown to work for other protein or peptide drugs as well. For example, oral administration of an anti-CD3 antibody has been shown to work in treatment of, for example, diabetes (Ishikawa et al. Diabetes. 2007 Aug;56(8):2103-9. Epub 2007 Apr 24), and autoimmune encephalomyelitis (Ochi et al. Nat Med. 2006 Jun;12(6):627-35. Epub 2006 May 21). Without wishing to be bound by a theory, we suggest

that this is possible via the gut associated lymphoid tissue (GALT). Accordingly, in some aspects of all the embodiments of the invention, the formulation of the peptides is oral formulation, and the methods are performed by administering the peptides orally.

[00245] Liquid dosage forms for oral administration of the compounds of the invention include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs.

[00246] In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

[00247] Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

[00248] Dosage forms for the topical or transdermal administration of one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants, which may be required.

[00249] The ointments, pastes, creams and gels may contain, in addition to an active compound of this invention, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof. Powders and sprays can contain, in addition to a compound of this invention, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

[00250] Transdermal patches have the added advantage of providing controlled delivery of the compounds (resolvins and/or protectins and/or precursors or analogues thereof) of the present invention to the body. Such dosage forms can be made by dissolving or dispersing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the active compound in a polymer matrix or gel. In another aspect, biodegradable or absorbable polymers can provide extended, often localized, release of polypeptide agents. The potential benefits of an increased half-life or extended release for a therapeutic agent are clear. A potential benefit of localized release is the ability to achieve much higher localized dosages or concentrations, for greater lengths of time, relative to broader systemic administration, with the potential to also avoid possible undesirable side effects that may occur with systemic administration.

[00251] Bioabsorbable polymeric matrix suitable for delivery of the one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof can be selected from a variety of synthetic bioabsorbable polymers, which are described extensively in the literature. Such synthetic bioabsorbable, biocompatible polymers, which may release proteins over several weeks or months can include, for example, poly- α -hydroxy acids (e.g. polylactides, polyglycolides and their copolymers), polyanhydrides, polyorthoesters, segmented block copolymers of polyethylene glycol and polybutylene terephthalate (Polyactive™), tyrosine derivative polymers or poly(ester-amides). Suitable bioabsorbable polymers to be used in manufacturing of drug delivery materials and implants are discussed e.g. in U.S. Pat. Nos. 4,968,317, 5,618,563, among others, and in "Biomedical Polymers" edited by S. W. Shalaby, Carl Hanser Verlag, Munich, Vienna, New York, 1994 and in many references cited in the above publications. The particular bioabsorbable polymer that should be selected will depend upon the particular patient that is being treated.

Gene therapy

[00252] One or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof can be effectively used in treatment by gene therapy. See, generally, for example, U.S. Pat. No. 5,399,346, which is incorporated herein by reference. The general principle is to introduce the polynucleotide into a target cell in a patient.

[00253] Entry into the cell is facilitated by suitable techniques known in the art such as providing the polynucleotide in the form of a suitable vector, or encapsulation of the polynucleotide in a liposome.

[00254] A desired mode of gene therapy is to provide the polynucleotide in such a way that it will replicate inside the cell, enhancing and prolonging the desired effect. Thus, the polynucleotide is operably linked to a suitable promoter, such as the natural promoter of the corresponding gene, a heterologous promoter that is intrinsically active in liver, neuronal, bone, muscle, skin, joint, or cartilage cells, or a heterologous promoter that can be induced by a suitable agent.

[00255] Expression vectors compatible with eukaryotic cells, preferably those compatible with vertebrate cells, can be used to produce recombinant constructs for the expression of one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof, including fusion proteins with one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof. Eukaryotic cell expression vectors are well known in the art and are available from several commercial sources. Typically, such vectors are provided containing convenient restriction sites for insertion of the desired DNA segment. These vectors can be viral vectors such as adenovirus, adeno-associated virus, pox virus such as an orthopox (vaccinia and attenuated vaccinia), avipox, lentivirus, murine moloney leukemia virus, etc. Alternatively, plasmid expression vectors can be used.

[00256] Viral vector systems which can be utilized in the present invention include, but are not limited to, (a) adenovirus vectors; (b) retrovirus vectors; (c) adeno-associated virus vectors; (d) herpes simplex virus vectors; (e) SV 40 vectors; (f) polyoma virus vectors; (g) papilloma virus vectors; (h) picornavirus vectors; (i) pox virus vectors such as an orthopox, e.g., vaccinia virus vectors or avipox, e.g. canary pox or fowl pox; and (j) a helper-dependent or gutless adenovirus. In a preferred embodiment, the vector is an adenovirus. Replication-defective viruses can also be advantageous.

[00257] The vector may or may not be incorporated into the cells genome. The constructs may include viral sequences for transfection, if desired. Alternatively, the construct may be incorporated into vectors capable of episomal replication, e.g. EPV and EBV vectors.

[00258] By "operably linked" is meant that a nucleic acid molecule and one or more regulatory sequences (e.g., a promoter) are connected in such a way as to permit expression and/or secretion of the product (e.g., a protein) of the nucleic acid molecule when the

appropriate molecules (e.g., transcriptional activator proteins) are bound to the regulatory sequences. Stated another way, the term "operatively linked" as used herein refers to the functional relationship of the nucleic acid sequences with regulatory sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of nucleic acid sequences, typically DNA, to a regulatory sequence or promoter region refers to the physical and functional relationship between the DNA and the regulatory sequence or promoter such that the transcription of such DNA is initiated from the regulatory sequence or promoter, by an RNA polymerase that specifically recognizes, binds and transcribes the DNA. In order to optimize expression and/or in vitro transcription, it may be necessary to modify the regulatory sequence for the expression of the nucleic acid or DNA in the cell type for which it is expressed. The desirability of, or need of, such modification may be empirically determined. An operatively linked polynucleotide which is to be expressed typically includes an appropriate start signal (e.g., ATG) and maintains the correct reading frame to permit expression of the polynucleotide sequence under the control of the expression control sequence, and production of the desired polypeptide encoded by the polynucleotide sequence.

[00259] As used herein, the terms "promoter" or "promoter region" or "promoter element" have been defined herein, refers to a segment of a nucleic acid sequence, typically but not limited to DNA or RNA or analogues thereof, that controls the transcription of the nucleic acid sequence to which it is operatively linked. The promoter region includes specific sequences that are sufficient for RNA polymerase recognition, binding and transcription initiation. This portion of the promoter region is referred to as the promoter. In addition, the promoter region includes sequences which modulate this recognition, binding and transcription initiation activity of RNA polymerase. These sequences may be ds-acting or may be responsive to trans-acting factors. Promoters, depending upon the nature of the regulation may be constitutive or regulated.

[00260] The term "regulatory sequences" is used interchangeably with "regulatory elements" herein refers element to a segment of nucleic acid, typically but not limited to DNA or RNA or analogues thereof, that modulates the transcription of the nucleic acid sequence to which it is operatively linked, and thus act as transcriptional modulators. Regulatory sequences modulate the expression of gene and/or nucleic acid sequence to which they are operatively linked. Regulatory sequence often comprise "regulatory elements" which are nucleic acid

sequences that are transcription binding domains and are recognized by the nucleic acid-binding domains of transcriptional proteins and/or transcription factors, repressors or enhancers etc. Typical regulatory sequences include, but are not limited to, transcriptional promoters, inducible promoters and transcriptional elements, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and sequences to control the termination of transcription and/or translation. Included in the term "regulatory elements" are nucleic acid sequences such as initiation signals, enhancers, and promoters, which induce or control transcription of protein coding sequences with which they are operatively linked. In some examples, transcription of a recombinant gene is under the control of a promoter sequence (or other transcriptional regulatory sequence) which controls the expression of the recombinant gene in a cell-type in which expression is intended. It will also be understood that the recombinant gene can be under the control of transcriptional regulatory sequences which are the same or which are different from those sequences which control transcription of the naturally-occurring form of a protein. In some instances the promoter sequence is recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required for initiating transcription of a specific gene.

[00261] Regulatory sequences can be a single regulatory sequence or multiple regulatory sequences, or modified regulatory sequences or fragments thereof. Modified regulatory sequences are regulatory sequences where the nucleic acid sequence has been changed or modified by some means, for example, but not limited to, mutation, methylation etc.

[00262] Regulatory sequences useful in the methods as disclosed herein are promoter elements which are sufficient to render promoter-dependent gene expression controllable for cell type- specific, tissue-specific or inducible by external signals or agents (e.g. enhancers or repressors); such elements may be located in the 5' or 3' regions of the native gene, or within an intron.

[00263] As used herein, the term "tissue-specific promoter" means a nucleic acid sequence that serves as a promoter, i.e., regulates expression of a selected nucleic acid sequence operably linked to the promoter, and which selectively affects expression of the selected nucleic acid sequence in specific cells of a tissue.

[00264] In some embodiments, it can be advantageous to direct expression of one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof in a tissue- or cell-specific manner. Muscle- specific expression can be achieved, for example, using the

skeletal muscle MKC promoter (as disclosed in U.S. Patent Application WO2007/100722, which is incorporated herein by reference), or other muscle- specific promoters, such as α -myosin heavy chain, myosin light chain-2 (which is specific for skeletal muscle (Shani et al., Nature, 314:283-86, 1985), gonadotrophic releasing hormone gene control region which is active in the hypothalamus (Mason et al, Science, 234:1372-78, 1986), and smooth muscle promoter SM22a, which are all commonly known in the art.

[00265] The term "constitutively active promoter" refers to a promoter of a gene which is expressed at all times within a given cell. Exemplary promoters for use in mammalian cells include cytomegalovirus (CMV), and for use in prokaryotic cells include the bacteriophage T7 and T3 promoters, and the like. The term "inducible promoter" refers to a promoter of a gene which can be expressed in response to a given signal, for example addition or reduction of an agent. Non-limiting examples of an inducible promoter are "tet-on" and "tet-off" promoters, or promoters that are regulated in a specific tissue type.

[00266] In a specific embodiment, viral vectors that contain nucleic acid sequences encoding the one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. More detail about retroviral vectors can be found in Boesen et al., Biotherapy 6:291-302 (1994), which describes the use of a retroviral vector to deliver the mdrl gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., J. Clin. Invest. 93:644-651 (1994); Kiem et al., Blood 83:1467-1473 (1994); Salmons and Gunzberg, Human Gene Therapy 4:129-141 (1993); and Grossman and Wilson, Curr. Opin. in Genetics and Devel. 3:110-114 (1993).

[00267] The production of a recombinant retroviral vector carrying a gene of interest is typically achieved in two stages. First, sequence encoding one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof can be inserted into a retroviral vector which contains the sequences necessary for the efficient expression of the metabolic regulators (including promoter and/or enhancer elements which can be provided by the viral long terminal repeats (LTRs) or by an internal promoter/enhancer and relevant splicing signals), sequences required for the efficient packaging of the viral RNA into infectious virions (e.g., a packaging signal (Psi), a tRNA primer binding site (-PBS), a 3' regulatory sequence required for reverse

transcription (+PBS)), and a viral LTRs). The LTRs contain sequences required for the association of viral genomic RNA, reverse transcriptase and integrase functions, and sequences involved in directing the expression of the genomic RNA to be packaged in viral particles.

[00268] Following the construction of the recombinant retroviral vector, the vector DNA is introduced into a packaging cell line. Packaging cell lines provide viral proteins required in trans for the packaging of viral genomic RNA into viral particles having the desired host range (e.g., the viral-encoded core (gag), polymerase (pol) and envelope (env) proteins). The host range is controlled, in part, by the type of envelope gene product expressed on the surface of the viral particle. Packaging cell lines can express ecotropic, amphotropic or xenotropic envelope gene products. Alternatively, the packaging cell line can lack sequences encoding a viral envelope (env) protein. In this case, the packaging cell line can package the viral genome into particles which lack a membrane-associated protein (e.g., an env protein). To produce viral particles containing a membrane-associated protein which permits entry of the virus into a cell, the packaging cell line containing the retroviral sequences can be transfected with sequences encoding a membrane-associated protein (e.g., the G protein of vesicular stomatitis virus (VSV)). The transfected packaging cell can then produce viral particles which contain the membrane-associated protein expressed by the transfected packaging cell line; these viral particles which contain viral genomic RNA derived from one virus encapsidated by the envelope proteins of another virus are said to be pseudotyped virus particles.

[00269] Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, *Current Opinion in Genetics and Development* 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., *Human Gene Therapy* 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Another preferred viral vector is a pox virus such as a vaccinia virus, for example an attenuated vaccinia such as Modified Virus Ankara (MVA) or NYVAC, an avipox such as fowl pox or canary pox. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., *Science* 252:431-434 (1991); Rosenfeld et al., *Cell* 68:143-155 (1992); Mastrangeli et al., *J. Clin. Invest.* 91:225-234 (1993); PCT Publication WO94/12649;

and Wang, et al., *Gene Therapy* 2:775-783 (1995). In another embodiment, lentiviral vectors are used, such as the HIV based vectors described in U.S. Patent Nos. 6,143,520; 5,665,557; and 5,981,276, which are herein incorporated by reference. Use of Adeno-associated virus (AAV) vectors is also contemplated (Walsh et al., *Proc. Soc. Exp. Biol. Med.* 204:289-300 (1993); and U.S. Pat. No. 5,436,146, which are incorporated herein by reference).

[00270] Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

[00271] U.S. Patent No. 5,676,954 (which is herein incorporated by reference) reports on the injection of genetic material, complexed with cationic liposome carriers, into mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provides methods for delivering DNA-cationic lipid complexes to mammals. Such cationic lipid complexes or nanoparticles can also be used to deliver protein.

[00272] A gene or nucleic acid sequence can be introduced into a target cell by any suitable method. For example, one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof constructs can be introduced into a cell by transfection (e.g., calcium phosphate or DEAE-dextran mediated transfection), lipofection, electroporation, microinjection (e.g., by direct injection of naked DNA), biolistics, infection with a viral vector containing a muscle related transgene, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, nuclear transfer, and the like. A nucleic acid encoding one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof can be introduced into cells by electroporation (see, e.g., Wong and Neumann, *Biochem. Biophys. Res. Commun.* 107:584-87 (1982)) and biolistics (e.g., a gene gun; Johnston and Tang, *Methods Cell Biol.* 43 Pt A:353-65 (1994); Fynan et al., *Proc. Natl. Acad. Sci. USA* 90:11478-82 (1993)).

[00273] In certain embodiments, a gene or nucleic acid sequence encoding one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof can be introduced into target cells by transfection or lipofection. Suitable agents for transfection or lipofection include, for example, calcium phosphate, DEAE dextran, lipofectin, lipfectamine, DIMRIE C, Superfect, and Effectin (Qiagen), unifectin, maxifectin, DOTMA, DOGS (Transfectam; dioctadecylamidoglycylspermine), DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine), DOTAP (1,2-dioleoyl-3- trimethylammonium propane), DDAB (dimethyl dioctadecylammonium bromide), DHDEAB (N,N-di-n-hexadecyl-N,N-dihydroxyethyl ammonium bromide), HDEAB (N- n-hexadecyl-N,N-dihydroxyethylammonium bromide), polybrene, poly(ethylenimine) (PEI), and the like. (See, e.g., Banerjee et al., *Med. Chem.* 42:4292-99 (1999); Godbey et al., *Gene Ther.* 6:1380-88 (1999); Kichler et al., *Gene Ther.* 5:855-60 (1998); Birchaa et al., *J. Pharm.* 183:195-207 (1999), incorporated herein by reference in their entireties).

[00274] Methods known in the art for the therapeutic delivery of agents such as proteins and/or nucleic acids can be used for the delivery of a polypeptide or nucleic acid encoding one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof, e.g., cellular transfection, gene therapy, direct administration with a delivery vehicle or pharmaceutically acceptable carrier, indirect delivery by providing recombinant cells comprising a nucleic acid encoding a targeting fusion polypeptide of the invention.

[00275] Various delivery systems are known and can be used to directly administer therapeutic polypeptides such as the one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof and/or a nucleic acid encoding one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, and receptor-mediated endocytosis (see, e.g., Wu and Wu, 1987, *J. Biol. Chem.* 262:4429-4432). Methods of introduction can be enteral or parenteral and include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, pulmonary, intranasal, intraocular, epidural, and oral routes. The agents may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

[00276] In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved, for example, and not by way of limitation, by local infusion during surgery, topical application, e.g., by injection, by means of a catheter, or by means of an implant, the implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, fibers, or commercial skin substitutes.

[00277] In another embodiment, the active agent can be delivered in a vesicle, in particular a liposome (see Langer (1990) *Science* 249:1527-1533). In yet another embodiment, the active agent can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer (1990) *supra*). In another embodiment, polymeric materials can be used (see Howard et al. (1989) *J. Neurosurg.* 71:105).

[00278] Thus, a wide variety of gene transfer/gene therapy vectors and constructs are known in the art. These vectors are readily adapted for use in the methods of the present invention. By the appropriate manipulation using recombinant DNA/molecular biology techniques to insert an operatively linked polypeptide encoding nucleic acid segment into the selected expression/delivery vector, many equivalent vectors for the practice of the methods described herein can be generated.

Other embodiments

[00279] From the foregoing description, it will be apparent that variations and modifications may be made to the invention described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

[00280] The disclosure also contemplates an article of manufacture, which is a labeled container for providing the one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof. An article of manufacture comprises packaging material and a pharmaceutical agent of the one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof, contained within the packaging material.

[00281] The pharmaceutical agent in an article of manufacture is any of the compositions of the present invention suitable for providing the one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof and formulated into a pharmaceutically acceptable form as described herein according to the disclosed indications. Thus, the composition can comprise the one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof or a DNA molecule which is capable of expressing such a peptide.

[00282] The article of manufacture contains an amount of pharmaceutical agent sufficient for use in treating a condition indicated herein, either in unit or multiple dosages. The packaging material comprises a label which indicates the use of the pharmaceutical agent contained therein.

[00283] The label can further include instructions for use and related information as may be required for marketing. The packaging material can include container(s) for storage of the pharmaceutical agent.

[00284] As used herein, the term packaging material refers to a material such as glass, plastic, paper, foil, and the like capable of holding within fixed means a pharmaceutical agent. Thus, for example, the packaging material can be plastic or glass vials, laminated envelopes and the like containers used to contain a pharmaceutical composition including the pharmaceutical agent.

[00285] In preferred embodiments, the packaging material includes a label that is a tangible expression describing the contents of the article of manufacture and the use of the pharmaceutical agent contained therein.

EXAMPLES

Example 1 - The Use of SP163M peptide for Treatment of Acute Myocardial Infarction

[00286] SP16 peptide is a short fragment derived from the circulating serum protein, alpha-1-antitrypsin (AAT). The US FDA has approved the use of three alpha 1-antitrypsin products derived from a human plasma: Prolastin, Zemaira, and Aralast.

[00287] As described herein, SP163M peptide contains the reported anti-inflammatory properties of the parental AAT. AAT has shown preliminary safety and efficacy in blocking the inflammatory reaction that occurs after AMI in a pilot clinical study conducted at Virginia Commonwealth University (VCU). Furthermore, SP163M has shown equal or better anti-inflammatory activity in preclinical models of AMI. We are collaborating with researchers at VCU to rapidly develop SP16 for treatment of AMI which represent a major unmet medical need in the US and worldwide.

[00288] Acute myocardial infarction (AMI) remains a major cause of morbidity and mortality in the US and worldwide. Despite current strategies for early reperfusion, many patients die early during the course, and those who survive are at risk for dying later from adverse cardiac remodeling, heart failure, and sudden death. Numerous publications emphasize

the correlation between the size of the infarct in AMI and the probability to progress to heart failure in the next 2-5 years post AMI.

[00289] Patients presenting with ST-segment elevation (STEMI) are at particularly high risk for adverse cardiac remodeling, heart failure, and in-hospital and long-term mortality. Although there have been considerable improvements in the treatment of STEMI, the reduction in early mortality has been associated with an increasing incidence of heart failure after STEMI.¹ This likely reflects more high risk patients surviving the index event as well as the aging of the population and the epidemics of hypertension and diabetes. Within 30 days of STEMI, more than 20% of survivors are diagnosed with heart failure, a disease associated with high morbidity, disability, and mortality.

[00290] Heart failure is indeed a major public health problem affecting approximately 5 million Americans with 500,000 new cases per year. In contrast to other cardiovascular disease, the incidence and prevalence of heart failure continue to increase and heart failure is now the leading cause of hospitalization for people aged 65 years, a segment of the population that is also rapidly growing. Although survival after the onset of heart failure is also improved, current therapies may slow but not halt the progression of the disease. With the limitations to functional capacity, the progressive symptoms of dyspnea and fatigue, the frequent hospital admissions and the economic consequences of lost productivity and increasing costs of medical care, heart failure imposes a significant burden on healthcare.

[00291] There is an urgent need to develop additional treatments to minimize the infarct size and prevent heart failure after AMI.² The current treatment in STEMI includes prompt reperfusion of the ischemic myocardium by restoration of the coronary artery patency (i.e. angioplasty or fibrinolysis), prevention of re-occlusion (i.e. antiplatelet and anticoagulants), and neuro-hormonal blockade (i.e. renin- angiotensin-aldosterone and adrenergic blockers). While each of these interventions provide incremental benefit and significantly reduce morbidity and mortality, the incidence of heart failure after STEMI has continued to rise, implying that the current treatment paradigm still misses one or more key pathophysiologic mechanisms

[00292] Determining the mechanisms by which unfavorable cardiac remodeling and heart failure progress despite optimal treatment is thus a critical step in the search for novel interventions, with the ultimate goal of reducing the incidence, burden, and mortality of heart failure after STEMI.

[00293] A close interplay exists between inflammation, adverse cardiac remodeling and heart failure after AMI. Acute myocardial ischemia and infarction initiate an intense inflammatory response within the myocardium.³ Leukocytes infiltrate the damaged myocardium to coordinate tissue repair and infarct healing, leading to newly formed vessels and reparative fibrosis. Thus, inflammation is necessary for infarct healing, but uncontrolled inflammation is responsible for further damage to the heart and non-functional healing which are the basis for adverse cardiac remodeling and heart failure. In experimental animal models, the degree of the inflammatory response determines adverse cardiac remodeling, independent of infarct size.³ In patients with AMI, the intensity of the inflammatory response, reflected in levels of circulating biomarkers, predicts adverse cardiac remodeling, heart failure and death.⁴ C- reactive protein (an acute phase reactant) are increased in AMI and predict outcome.

[00294] Modulation of the inflammatory response therefore represents a target for intervention. While previous attempts to modulate the inflammatory response have failed, we propose a novel and substantially different approach to modulating the inflammatory response using SP163M peptide a 17-mer modified derivative of Alpha-1 Anti- Trypsin (AAT).⁵ AAT is a naturally occurring anti-inflammatory protein abundant in the plasma, exerting powerful cyto-protective effects in endothelial cells, cardiomyocytes and fibroblasts.⁵

[00295] In an experimental model of acute myocardial infarction (AMI), AAT administered at the onset of ischemia or at time of reperfusion led to a significantly smaller infarct size and more favorable cardiac healing and remodeling (Figure 3A-3D).⁶

[00296] While there may be more than one mechanism by which AAT improves cardiac function during AMI, an inhibition of the production of Interleukin-1 (IL-1) is proposed.⁶⁻⁷ This is relevant because IL-1 inhibition has been shown to be safe and efficacious in limiting myocardial damage during AMI both in experimental studies and in 2 small pilot clinical trials that were completed at VCU.⁸⁻¹⁰

[00297] In order to examine the effects of SP163M in AMI, the peptide was tested in the same mouse model previously used for AAT. Briefly, adult male ICR mice underwent coronary artery ligation inducing myocardial ischemia for 30 minutes followed by reperfusion. SP16 at 100 µg dose or matching volume of NaCl 0.9% solution was given at time of reperfusion. SP16 significantly reduces by >50% the myocardial infarct size (Figure 4A), measured as percentage of infarcted left ventricle (appearing white at the triphenyltetrazolium chloride stain) on the whole left ventricle, with viable myocardium appearing bright red. The

plasma levels of cardiac specific troponin I, a biomarker of myocardial necrosis, was also significantly reduced by SP163M treatment (Figure 4B). The cardioprotective effects of SP16 translated in preservation of the left ventricular systolic function, measured as left ventricular fractional shortening using transthoracic-echocardiography (Figure 4C).

[00298] *Protective effects of SP163M in experimental acute myocardial infarction in the mouse*

[00299] Ten-weeks-old male ICR (CD1) mice underwent surgical coronary artery ligation for 30 minutes, followed by reperfusion for 24 hours, to simulate the scenario of acute myocardial infarction (AMI) treated with reperfusion therapies. A subgroup of mice underwent sham surgery in which the coronary artery was identified and isolated but not ligated.

[00300] SP163M -active peptide- or a control solution -vehicle- were administered at a dose of 100 µg/mouse i.p. at reperfusion

[00301] Infarct size was measured at pathology with triphenyltetrazolium chloride(TTC)/Evans blue staining, and by measuring plasma troponin I levels at 24 hours. Evans blue was retrogradely injected through the aorta after re-ligation of the coronary artery knot to identify the non-risk myocardium (blue). TTC was used to stain viable myocardium (red).

[00302] Echocardiography was used to assess left ventricular (LV) fractional shortening, as a measure of systolic function in vivo.

[00303] Each group included at least 6 mice.

[00304] *Protective effects of SP163M administered within 30 minutes of reperfusion in experimental AMI in the mouse*

[00305] The option of delaying treatment with SP163M for additional 30 minutes after reperfusion was examined. A control peptide SP34 which has 16/17 amino acids identical in sequence as SP163M but in a scrambled order was also tested.

[00306] Ten-weeks-old male ICR (CD1) mice underwent surgical coronary artery ligation for 30 minutes, followed by reperfusion for 24 hours, to simulate the scenario of AMI treated with reperfusion therapies.

[00307] SP163M -active peptide- or SP34 -control peptide- were administered at a dose of 100 µg/mouse i.p. at the moment of the reperfusion or with 30' of delay, to simulate a clinical scenario in which a delay between reperfusion and pharmacologic therapy is likely to occur.

[00308] Infarct size was measured with TTC/Evans blue staining. Echocardiography was used to assess left ventricular (LV) fractional shortening, as a measure of systolic function in vivo. Each group included at least 6 mice.

[00309] SP163M given with 30' of reperfusion significantly reduced infarct size (Figure 5A) and preservation of LV fractional shortening (Figure 5B).

[00310] *SP163M provides a dose-dependent reduction in infarct size in experimental AMI in the mouse*

[00311] The cardio-protective capacity of SP163M at 10 µg dose to the 100 µg dose was compared. The results are depicted in Figure 6 showing that even a reduction of SP163M dose by 90% was shown to be effective as measured by infarct size and LV systolic function (Figure 6 A and 6B, respectively).

[00312] Ten-weeks-old ICR mice underwent surgical coronary artery ligation for 30 minutes, followed by reperfusion for 24 hours. SP16 was administered at 2 different doses (10 or 100 µg) with 30' of delay. The control peptide SP34 (100 µg) or the vehicle solution were used as controls.

[00313] Infarct size was measured with TTC/Evans blue staining. Transthoracic echocardiography was used to assess left ventricular (LV) fractional shortening. Each group included at least 6 mice.

[00314] SP163M given with 30' of reperfusion reduces infarct size (Figure 6A) and preserves LV fractional shortening (Figure 6B) in a dose-dependent fashion.

[00315] *SP163M has no effects on cardiac contractility in the healthy mouse*

[00316] To test the effect of SP163M on heart contractility the following study was conducted. SP163M peptide was shown to not effect heart contractility which adds to the safety profile of this peptide drug (Figure 7). SP34 is a peptide with a scrambled sequence of SP16 and is used as a control peptide.

[00317] Ten-weeks-old ICR mice were treated with SP163M (100 µg/mouse i.p.), the control peptide SP34 (100 µg/mouse) or the vehicle solution. Echocardiography was used to assess left ventricular (LV) fractional shortening at baseline and after 180 minutes.

[00318] Cardiac contractility reserve was tested with isoproterenol challenge (10 ng/kg i.p.), measuring the increase in LV fractional shortening within 5' of treatment. Each group included at least 6 mice.

[00319] SP163M does not alter cardiac systolic function at rest (Figure 7, left panel) or after isoproterenol challenge (contractile reserve)(Figure 7, right panel).

[00320] *Discussion*

[00321] In addition to being its anti inflammatory effect, SP163M is also cardio-protective. It reduces the size of the infarct through rescuing the cardiac cells from death upon reperfusion IL1 antagonist tested in AMI and showed a clear anti inflammatory effect but no reduction in infarct size (not cardio-protective). SP163M cell-protective effect manifested in AMI in a decrease of infarct can also be applicable to other injuries resulting in cell death such as other ischemia situations, stroke, traumatic brain injury and toxic shocks. It is contemplated herein that SP163M can permit treatment of Acute Myocardial Infarction, gout, stroke, complications associated with heart surgery, traumatic brain injury, any other disease with “cytokine storm” involvement.

[00322] REFERENCES

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Example 2

[00323] Met was replaced by Nle to enhance peptide stability, e.g., in the peptide of SEQ ID NO: 57 - Ac-Val-Lys-Phe-Asn-Lys-Pro-Phe-Val-Phe-Leu-*Nle*-Ile-Glu-Gln-Asn-Thr-Lys-NH₂ (SEQ ID NO: 57). Methionine can be oxidized.

Example 3 – LRP1 mediates SP16-induced cardioprotection

[00324] SP163M is demonstrated herein to bind to LRP1 (Low density lipoprotein receptor-related protein) and to be an agonist (as was shown for the parental AAT) of LRP1. It is further shown that blocking LRP1 eliminates the cardioprotective effects of SP163M and AAT.

[00325] *Introduction*

[00326] LRP1 is a receptor responsible for plasma clearance of SECs (Serpin Enzyme Complexes accumulating during an inflammatory response). Thus, LRP1's role is to block and discontinue inflammation. LRP1 can act via direct modulation of key inflammatory pathways; e.g. NFκB, IRF-3, and AP-1 (e.g., ERK p42/p44, p38 and JNK).

[00327] In murine peritoneal macrophages, LRP1 down regulates the LPS driven inflammatory response through intracellular interaction with IRF-3. Additionally, LRP1 regulates inflammatory cytokines, impacts phagocytosis and cellular migration, and Treg profile. Serpins, including, e.g., the peptides of SEQ ID NO: 1 and 57, can bind to Cluster II or IV of LRP1 via a highly conserved pentapeptide. See Figure 12A. Accordingly, SP163M can

induce a cascade leading to down-regulation of inflammatory response through cell signaling, modulation of cytokines, decrease of CD4 T cells, and Th17 cells.

[00328] Modulation of LRP1 is implicated in a variety of inflammatory and auto-immune diseases. Diseases such as neurodegenerative diseases, atherosclerosis, and type II diabetes. Acute myocardial ischemia and reperfusion leads to myocardial necrosis and inflammation, which, if left unresolved can lead to tissue injury and heart failure. Ischemic injury is the hallmark of AMI. Reperfusion, although beneficial in reducing the overall infarct size, is associated with more injury. The inflammatory response to ischemia-reperfusion injury, while promoting infarct healing, induces further loss of viable myocardium leading to dysfunctional scar formation. A large infarct size, an intense inflammatory response, and the formation of dysfunctional scar, all represent the substrate for heart failure following AMI. Prompt resolution of the inflammatory response, on the other hand, is associated with more favorable healing and reduced incidence of heart failure.

[00329] Although not wishing to be bound by theory, it is proposed that the inflammatory response to tissue injury following AMI leads to the recruitment of plasma SERPINs at site of injury, inhibition of leukocyte serine proteases, and signaling toward resolution of the inflammatory response through LRP1. It is therefore proposed that administration of exogenous plasma-derived SERPINs early during AMI will lead to inhibition of the inflammatory injury through LRP1 resulting in a cardioprotective effect and more prompt resolution of the inflammation. Furthermore, it is proposed that administration of a synthetic small peptide derived from the C3terminus of SERPINs will provide a powerful LRP1 mediated cardioprotective signal, without inhibiting plasma serine proteases. See Figure 11.

[00330] *SP163M increases LRP1 expression on murine macrophages*

[00331] SP163M is demonstrated herein to increase expression of LRP1 on Raw264.7 murine macrophages in a dose-dependent manner (Figure 10). Macrophages were plated 0.2×10^6 in a 24 well plate and grown to 70% confluence. Cells were treated with vehicle (red), SP16 at 50 (Purple), 100 (Green) or 200ug/ml (Blue), scrambled control (Orange) or COG133 (ApoE peptide) (Pink) for 3 hrs before harvesting with cold PBS 5mM EDTA. Samples were labeled with anti-LRP1 (abcam ab92544) and stained with Alexa Fluor™ 488 rabbit secondary (invitrogen) and then analyzed by flow cytometry (Guava easyCyte™ flow cytometer). The mean fluorescent intensity (MFI) normalized to the control is shown. (For each sample >10,000 events were collected).

[00332] *SP163M binds to LRP1*

[00333] An *in vitro* assay was performed to examine SP163M binding to LRP1. To perform the assay, recombinant LRP1 protein (1 µg/ml) (R&D Systems, Minneapolis, MN) was incubated on ice in the presence of biotin-SP163M (1-5 µg/ml) at 4°C for 1 hour. This mixture was then incubated with 1 mg/ml M280 Streptavidin Dynabeads (Invitrogen, Carlsbad, CA) for 1.5 hours at 4°C. Dynabeads were washed in PBS + 0.1% Tween20 and boiled in SDS loading buffer for 5 minutes. The supernatants were separated on NuPAGE 4-20% Bis-Tris gels (Invitrogen) and transferred to nitrocellulose membranes. The membranes were probed with anti-LRP1 (LRP-1 Cluster II Affinity Purified Polyclonal Ab, R&D, Minneapolis, MN) and incubated overnight at 4°C. The membranes were then washed 3 times with TBS-T and incubated with secondary HRP-coupled anti-goat diluted 1:10,000 in 5% milk TBS-T. The western blots were visualized by chemiluminescence using SuperSignal West Femto Maximum Sensitivity Substrate kit (ThermoScientific) and a Bio Rad Molecular Imager ChemiDoc XRS system (Bio-Rad, Hercules, CA).

[00334] Figure 12B shows that LRP1 binds SP163M at concentrations of 1 µg and 5 µg. Meanwhile LRP1 did not bind a control, SP34, peptide.

[00335] *SP163M is an LRP1 agonist*

[00336] THP1-XBlue™-MD2-CD14 cells (InvivoGen, San Diego, CA) were used in an *in vitro* assay to examine whether SP163M is an agonist of LRP1. The THP1-XBlue™-MD2-CD14 cells were maintained in RPMI 1640 medium (Thermo Scientific) supplemented with 10% heat inactivated FBS, 1% Pen-Strep, 100ug/ml Normocin™, 200ug/ml Zeocin™, and 250µg/ml of G418. The cell lines were maintained in 5% CO₂ at 37°C and in accordance with the distributor's guidelines.

[00337] The THP1-XBlue™-MD2-CD14 cells were seeded at 1×10^5 cells/well in a 96-well cell culture plate. The cells were treated with peptide or a scrambled control peptide (100 µg/ml), for 30 minutes prior to the addition of LPS (5 ng/ml) or GP96 (100 pM). After an 18-24 hour incubation at 37°C, 20 µl of supernatant was transferred to 180µl of QUANTI-Blue™ SEAP detection medium. NF-κB inducible SEAP levels were detected by measuring the absorbance. In experiments blocking LRP1, the anti-LRP1 antibody (125 µg/ml) (clone 5A6, Molecular Innovations, Novi, MI) or Receptor-Associated Protein (RAP) (1 µM) (Molecular Innovations) were used 30 minutes prior to the addition of the peptide.

[00338] As shown in Figure 13, SP163M inhibits NF- κ B signaling induced by LPS (Figure 13A) and GP96 (Figure 13B). Treatment with an LRP antibody or RAP (a co-factor leading to LRP1 downregulation, limits SP163M-related inhibition in both LPS and GP96 treated cells.

[00339] *LRP1 mediates SP163M-induced cardioprotection*

[00340] To examine the whether LRP1 mediates the cardioprotective effect of SP163M, groups of mice were treated with an LRP1 blocking antibody prior to experimental AMI. The resulting infarct size and LV systolic function were measured

[00341] Adult out-bred male CD1 mice (10 weeks of age) supplied by Harlan Sprague Dawley (Indianapolis, IN) were used in this study. A single operator (S.T.) skilled in coronary artery surgical ligation in mice performed all surgeries. Experimental AMI was induced by transient coronary artery ligation of the left anterior descending coronary artery for 30 minutes to induce ischemia of the anterior wall and the apex (visible as pallor) followed by reperfusion, and leading to an infarct involving approximately 15% of the left ventricle [1].

[00342] At the time of the surgeries, the mice were deeply sedated with sodium pentobarbital (70-100 mg/kg), intubated and placed in the right lateral decubitus. A left thoracotomy was performed followed by pericardiectomy and ligation of the proximal left coronary artery, followed by reperfusion. After closure of the thoracic access, the mice were left to recover for up to 1 week with unlimited access to food and water. Only the mice that showed evidence of ischemia at visual inspection during surgery and involving the whole apex were randomly assigned to different post treatment groups by an investigator (A.G.M) not involved in the adjudication of the endpoints of interest. The experiments were conducted under the guidelines of laboratory animals for biomedical research published by National Institutes of Health (revised 2011). The study protocol was approved by the Institutional Animal Care and Use Committee of the Virginia Commonwealth University.

[00343] Mice that received LRP1 blocking antibody as a pretreatment were administered the antibody 16 hours prior to the surgery to allow adequate time for antibody binding to the target receptor. Mice were randomly assigned to various post-surgery treatment groups, where the post-surgery treatment was given as a single intraperitoneal administration immediately after reperfusion. The post-surgery treatment groups were: SP163M 100 μ g, matching volume of vehicle to SP163M, LRP1 blocking antibody (AB) clone 5A6 3mg/kg (Molecular Innovations), LRP1-AB and SP163M 100 μ g, plasma derived alpha-1 antitrypsin (AAT) 60

mg/kg (Aralast NP, Baxter, Deerfield, IL, USA), LRP1-AB and AAT. Each group included 5 to 8 mice.

[00344] Infarct size (reported in Figure 14A) was measured as previously described [1]. A subgroup of mice were sacrificed 24 hours after surgery and the hearts were quickly removed and mounted on a Langendorff apparatus, where the coronary arteries were antegradely perfused with phosphate buffered saline (PBS) 1X pH7.4, containing Heparin (40 U/ml). After the blood was washed out, 10% triphenyltetrazolium chloride (Sigma Aldrich) in PBS 1X was perfused, followed by the tying of the ligature and infusion of 1% Phthalo blue dye (Quantum Ink, Louisville, KY, USA) 5 mM adenosine in PBS1X was injected as a bolus into the aorta until most of the heart turned blue. After, the hearts were then removed from the Langendorff apparatus, frozen, and cut into 6 transverse slices of equal thickness, about 1 mm, from apex to base. The infarcted tissue (appearing white) and the viable tissue (bright red) were measured by computer morphometry using Image Pro Plus 6.0 software (Media Cybernetics, Silver Spring, MD). Infarct size is reported as % of the left ventricle.

[00345] LV systolic function (reported in Figure 14B) was determined as well. The mice underwent transthoracic echocardiography at baseline (before surgery) under mild anesthesia with sodium pentobarbital (30-50 mg/kg) and at 24 hours. Echocardiography was performed with the Vevo770 imaging system (VisualSonics Inc, Toronto, Ontario, Canada) and a 30-MHz probe [1, 2]. The heart was visualized in B-mode from parasternal short axis and apical views. We measured the left ventricular (LV) end-diastolic and end-systolic areas at B-Mode and the LV end-diastolic diameter (LVEDD), LV end-systolic diameters (LVESD) at M-Mode. LV fractional shortening (FS) and LV ejection fraction (EF) were calculated. A subgroup of mice underwent a repeated echocardiography at 7 days to measure the infarct size (number of segments with akinesis [2]) and the left ventricular systolic function.

[00346] All results are expressed as mean and standard error. Comparisons between 3 or more groups were performed using analysis of variance (ANOVA), followed by a T test for unpaired data to compare each treatment group with the respective control. Kaplan Meyer survival curves for SP16 and vehicle after LPS challenge were compared using the Log-rank test. We used Statistical Package for Social Sciences (SPSS version 22.0, IBM inc., New York, NY) for all analysis. A P value <0.05 was considered significant.

[00347] *Results.* Treatment of the mice with blocking LRP1 antibody eliminated the protective effects of both SP163M and plasma-derived AAT.

[00348] REFERENCES

1. Toldo S, Seropian IM, Mezzaroma E, Van Tassell BW, Salloum FN, Lewis EC, Voelkel N, Dinarello CA, Abbate A: **Alpha-1 antitrypsin inhibits caspase-1 and protects from acute myocardial ischemia-reperfusion injury.** Journal of molecular and cellular cardiology 2011, 51(2):244-251.
2. Seropian IM, Abbate A, Toldo S, Harrington J, Smithson L, Ockaili R, Mezzaroma E, Damilano F, Hirsch E, Van Tassell BW: **Pharmacologic Inhibition of Phosphoinositide 3-Kinase Gamma (PI3K γ) Promotes Infarct Resorption and Prevents Adverse Cardiac Remodeling After Myocardial Infarction in Mice.** Journal of cardiovascular pharmacology 2010, 56(6):651-658.

CLAIMS

We claim:

1. A method of treating a disease associated with a cytokine storm, comprising administering to a human subject affected with the disease a pharmaceutical composition comprising a peptide selected from the group consisting of:
 - (i) a peptide comprising the amino acid sequence
X1-Z1-F-N-K-P-F-X2-Z2-X3-Z3-Q (SEQ ID NO: 2), wherein
X1 is V or L;
X2 is V, L, Nle, or M;
X3 is M, Nle, I or V;
Z1 is any amino acid;
Z2 is a sequence of any two amino acids; and
Z3 is a sequence any five amino acids, and wherein the peptide comprises 37 or fewer amino acids;
 - (j) a peptide comprising the amino acid sequence VKFNKPFVFLMIEQNTK (SEQ ID NO: 1);
 - (k) a peptide consisting essentially of the amino acid sequence
X1-Z1-F-N-X2-P-F-X3-Z2-X4-Z3-X5 (SEQ ID NO: 3), wherein
X1 is V or L;
X2 is K or R;
X3 is V, L, Nle, or M;
X4 is M, Nle, I or V;
X5 is K or Q;
Z1 is any amino acid;
Z2 is a sequence of any two amino acids; and
Z3 is a sequence any five amino acids;
 - (l) a peptide consisting essentially of the amino acid sequence RFNRPFLR (SEQ ID NO: 4).
 - (m) a peptide consisting essentially of the amino acid sequence of
RRRFNRPFLRRR (SEQ ID NO: 8).

- (n) a peptide consisting essentially of the amino acid sequence of VKFNKPFVFLMIEQNTK (SEQ ID NO: 1);
 - (o) a peptide consisting essentially of the amino acid sequence of FNRPFLL (SEQ ID NO: 10); and
 - (p) a peptide comprising SEQ ID NO: 57.
2. The method of claim 1, wherein the disease associated with a cytokine storm is selected from the group consisting of:
- acute myocardial infarction (AMI); gout; stroke; heart surgery complications; and
 - traumatic brain injury.
3. A method of reducing infarct size in a human subject in need of treatment therefor, comprising administering to the human subject a pharmaceutical composition comprising a peptide selected from the group consisting of:
- (a) a peptide comprising the amino acid sequence X1-Z1-F-N-K-P-F-X2-Z2-X3-Z3-Q (SEQ ID NO: 2), wherein
 - X1 is V or L;
 - X2 is V, L, Nle, or M;
 - X3 is M, Nle, I or V;
 - Z1 is any amino acid;
 - Z2 is a sequence of any two amino acids; and
 - Z3 is a sequence any five amino acids, and wherein the peptide comprises 37 or fewer amino acids;
 - (b) a peptide comprising the amino acid sequence VKFNKPFVFLMIEQNTK (SEQ ID NO: 1);
 - (c) a peptide consisting essentially of the amino acid sequence X1-Z1-F-N-X2-P-F-X3-Z2-X4-Z3-X5 (SEQ ID NO: 3), wherein
 - X1 is V or L;
 - X2 is K or R;
 - X3 is V, L, Nle, or M;
 - X4 is M, Nle, I or V;
 - X5 is K or Q;
 - Z1 is any amino acid;
 - Z2 is a sequence of any two amino acids; and

- Z3 is a sequence any five amino acids;
- (d) a peptide consisting essentially of the amino acid sequence RFNRPFLR (SEQ ID NO: 4).
 - (e) a peptide consisting essentially of the amino acid sequence of RRRFNRPFLRRR (SEQ ID NO: 8).
 - (f) a peptide consisting essentially of the amino acid sequence of VKFNKPFVFLMIEQNTK (SEQ ID NO: 1);
 - (g) a peptide consisting essentially of the amino acid sequence of FNRPFLL (SEQ ID NO: 10); and
 - (h) a peptide comprising SEQ ID NO: 57.
4. The method of claim 3, wherein the human subject in need of a reduction in infarct size is a subject in need of treatment for a disease selected from the group consisting of:
- acute myocardial infarction (AMI); ischemia; stroke; traumatic brain injury; and
 - toxic shock.
5. A method of treating acute myocardial infarction (AMI), comprising administering to a human subject affected with AMI a pharmaceutical composition comprising a peptide selected from the group consisting of:
- (a) a peptide comprising the amino acid sequence X1-Z1-F-N-K-P-F-X2-Z2-X3-Z3-Q (SEQ ID NO: 2), wherein
 - X1 is V or L;
 - X2 is V, L, Nle, or M;
 - X3 is M, Nle, I or V;
 - Z1 is any amino acid;
 - Z2 is a sequence of any two amino acids; and
 - Z3 is a sequence any five amino acids, and wherein the peptide comprises 37 or fewer amino acids;
 - (b) a peptide comprising the amino acid sequence VKFNKPFVFLMIEQNTK (SEQ ID NO: 1);
 - (c) a peptide consisting essentially of the amino acid sequence X1-Z1-F-N-X2-P-F-X3-Z2-X4-Z3-X5 (SEQ ID NO: 3), wherein
 - X1 is V or L;
 - X2 is K or R;

X3 is V, L, Nle, or M;

X4 is M, Nle, I or V;

X5 is K or Q;

Z1 is any amino acid;

Z2 is a sequence of any two amino acids; and

Z3 is a sequence any five amino acids;

(d) a peptide consisting essentially of the amino acid sequence RFNRPFLR (SEQ ID NO: 4).

(e) a peptide consisting essentially of the amino acid sequence of RRRFNRPFLRRR (SEQ ID NO: 8).

(f) a peptide consisting essentially of the amino acid sequence of VKFNKPFVFLMIEQNTK (SEQ ID NO: 1);

(g) a peptide consisting essentially of the amino acid sequence of FNRPFLL (SEQ ID NO: 10);

(h) a peptide comprising SEQ ID NO: 57.

6. The method of any of claims 1-5, wherein the pharmaceutical composition is an oral formulation.
7. The method of any of claims 1-6, wherein the peptide further comprises at least one second peptide or protein.
8. The method of claim 7, wherein the at least one second protein or peptide is attached to the peptide as a fusion peptide.
9. The method of any of claims 7-8, wherein the at least one second peptide or protein is an epitope tag or a half-life extender or both.
10. The method of any of claims 1-9, wherein the peptide comprises one or more D-amino acids.
11. The method of any of claims 1-10, wherein the peptide causes a 75% decrease in serum TNF- α levels when administered in an effective amount to a human subject.
12. The method of any of claims 1-11, wherein the peptide consists of 35 amino acid residues or fewer.
13. The method of any of claims 1-12, wherein the peptide consists of 22 amino acid residues or fewer.

14. The method of any of claims 1-13, wherein the peptide consists of 21 amino acid residues or fewer.
15. The method of any of claims 1-14, wherein the composition further comprises a pharmaceutically acceptable carrier.
16. The method of any of claims 1-15, wherein the subject does not have, does not have symptoms of, or is not diagnosed as having a condition selected from the group consisting of:
 - type II diabetes, lupus, graft versus host disease, uveitis, eczema, psoriasis, cystic fibrosis, rheumatoid arthritis, acute radiation syndrome, burn patients, inflammatory bowel disease, type I diabetes, and hyperglycemia.

SERPIN C-TERMINAL PEPTIDES

HUMAN AAT C36	1	S	I	P	P	E		V	K	F	N	K	P	F	V	F	L	M	I	E	Q	N	T	K	S	P	L	F	M	G	K	V	V	N	P	T	Q	K			
HUMAN KALLIST ATIN	2	S	A	Q	T	N	R	H	I	L	R	F	N	R	P	F	L	V	V	I	F	S	T	S	T	Q	S	V	L	F	L	G	K	V	V	D	P	T	K	P	
(C39) HUMAN ANTICHY MOTRYP	3	S	A	L	V	E	T	R	T	I	V	R	F	N	R	P	F	L	M	I	I	V	P	T	D	T	Q	N	I	F	F	M	S	K	V	T	N	P	K	Q	A
SI n (C40)																																									
RAT SERPINA3 M(C38)	4	S	G	R	P	P	M	I		V	W	F	N	R	P	F	L	I	A	V	S	H	T	H	G	Q	T	I	L	F	M	A	K	V	I	N	P	V	G	A	
RAT SERPINA3 K(C38)	5	K	S	L	P	Q	T	I	P	L	L	N	F	N	R	P	F	M	L	V	I	T	D	D	N	G	Q	S	V	F	F	M	G	K	V	T	N	P	M		
HUMAN HYBRID 1	6	S	A	Q	V	E	T	I		V	R	F	N	R	P	F	L	V	I	I	V	S	T	N	T	Q															
HUMAN- RAT HYBRID 1	7	S	I	P	P	Q	M	I		V	W	F	N	R	P	F	L	I	A	I	S	H	T	H	T	Q															

FIG. 1

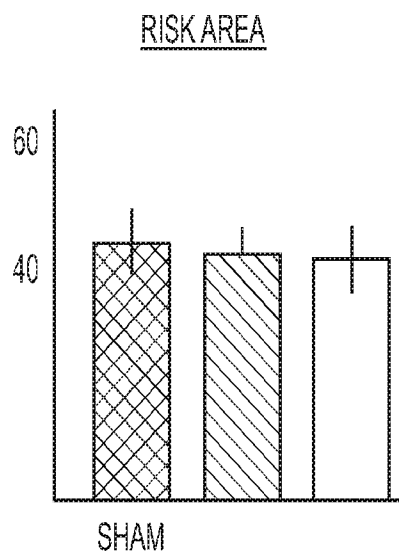
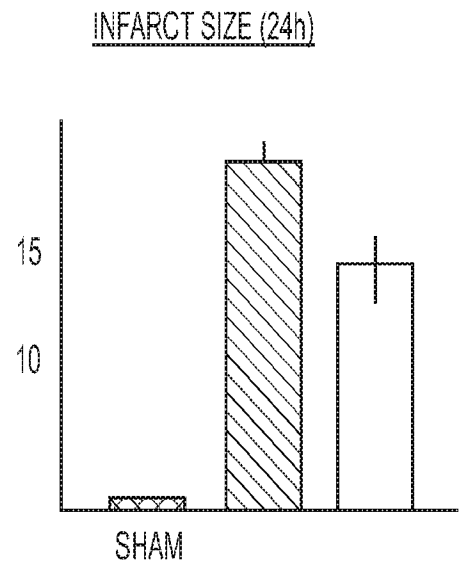
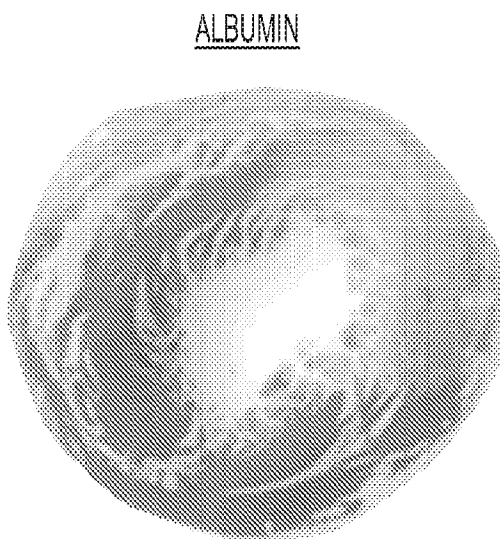
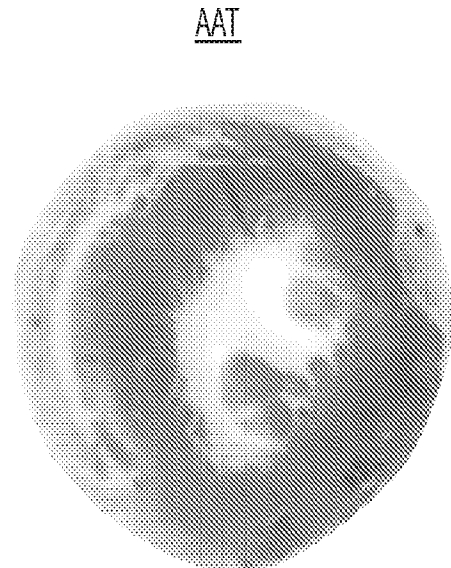
2/14

PEPTIDES DERIVED BY TRUNCATION

HUMAN AAT C36	8	S	I	P	P	E		V	K	F	N	K	P	F	V	F	L	M	I	E	Q	N	T	K							
HUMAN KALLISTATIN (C39)	9	S	A	Q	T	N	R	H	I	L	R	F	N	R	P	F	L	V	V	I	F	S	T	S	T	Q					
HUMAN ANTICHYMOTRYPSIN (C40)	10	S	A	L	V	E	T	R	T	I	V	R	F	N	R	P	F	L	M	I	V	P	T	D	T	Q					
RAT SERPINA3K (C38)	11	K	S	L	P	Q	T	I	P	L	L	N	F	N	R	P	F	M	L	V	I	T	D	D	N	G	Q				
HUMAN AAT C36	12										I	P	P	E	V	K	F	N	K	P	F	V	F	L	M	I	E	Q	N	T	K
HUMAN KALLISTATIN (C39)	13										N	R	H	I	L	R	F	N	R	P	F	L	V	V	I	F	S	T	S	T	Q
HUMAN ANTICHYMOTRYPSIN (C40)	14										T	R	T	I	V	R	F	N	R	P	F	L	M	I	V	P	T	D	T	Q	
RAT SERPINA3K (C38)	15										T	I	P	L	L	N	F	N	R	P	F	M	L	V	I	T	D	D	N	G	Q
C36 CORE SEQUENCE, LONG	16																														
C36 CORE SEQUENCE, SHORT	17																														
HUMAN AAT C36	18	S	I	P	P	E		V	K	A	A	A	A	A	A	F	L	M	I	E	Q	N	T	K							
HUMAN AAT C36	19																														
HUMAN AAT C36	20																														
HUMAN AAT C36	21																														
HUMAN AAT C36	22																														
HUMAN KALLISTATIN (C39)	23																														
HUMAN KALLISTATIN (C39)	24																														
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HUMAN ANTICHYMOTRYPSIN (C40)	32																														
HUMAN AAT C36	33																														
SCRAMBLED	34																														
CORE	35																														
CORE	36																														

FIG. 2

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**FIG. 3A****FIG. 3B****FIG.3C****FIG.3D**

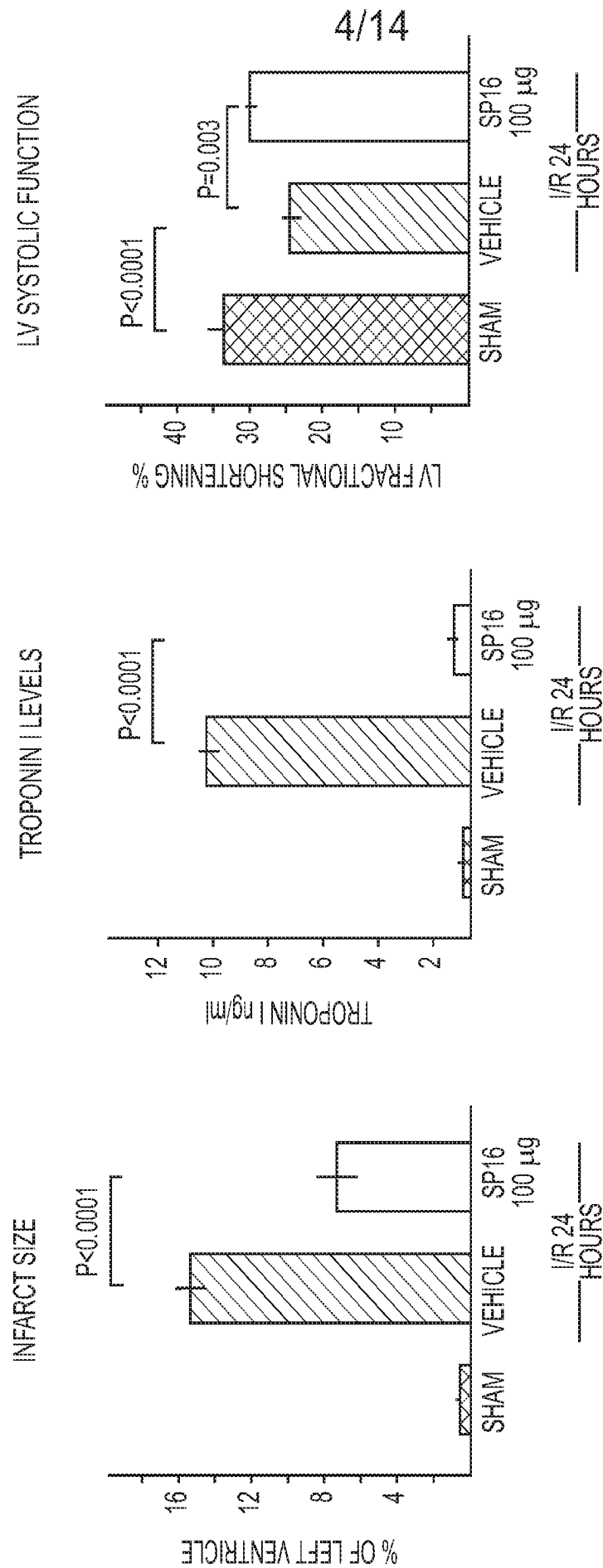
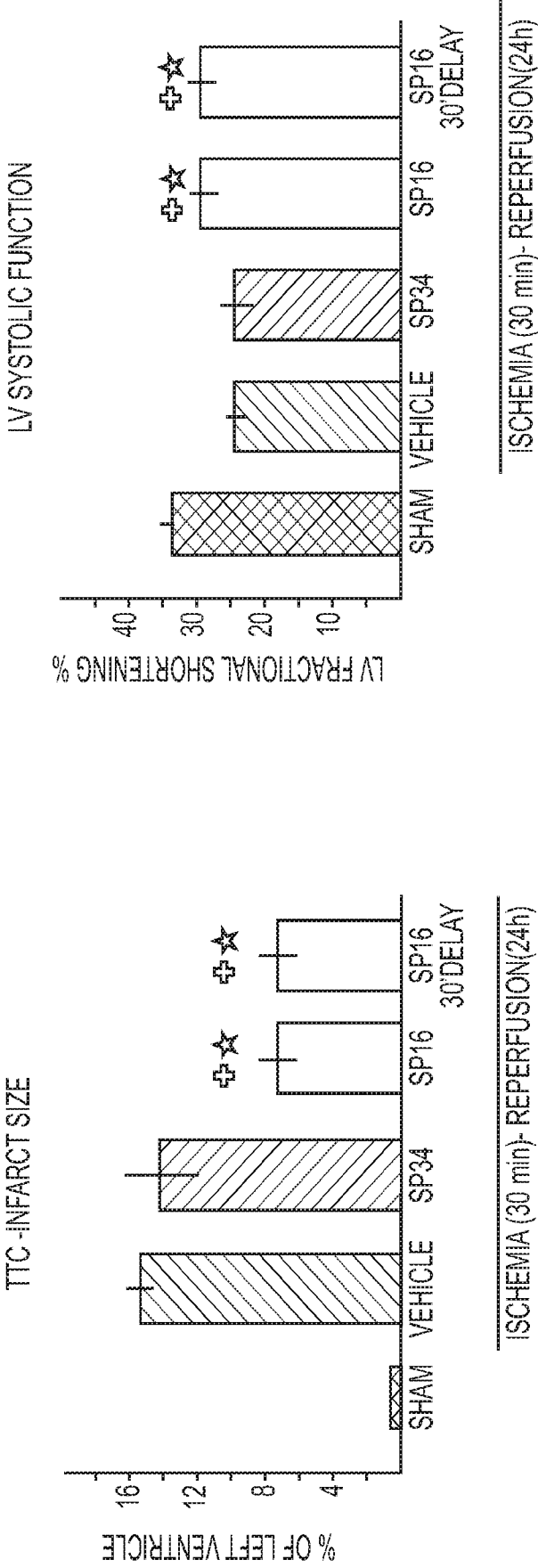


FIG. 4A

FIG. 4B

FIG. 4C



⊕ P<0.001 VS VEHICLE; ★ P<0.001 VS SP34

FIG. 5A

⊕ P<0.01 VS VEHICLE; ★ P<0.05 VS SP34

FIG. 5B

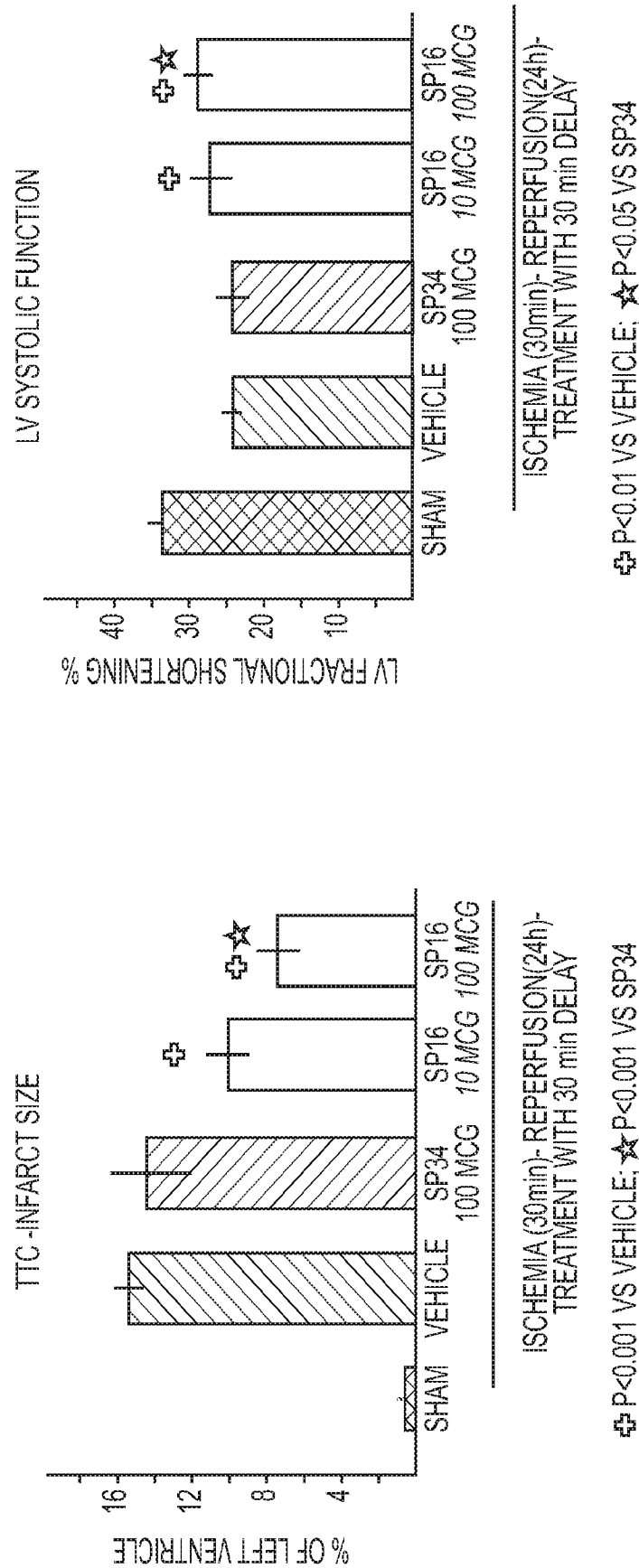


FIG. 6A

FIG. 6B

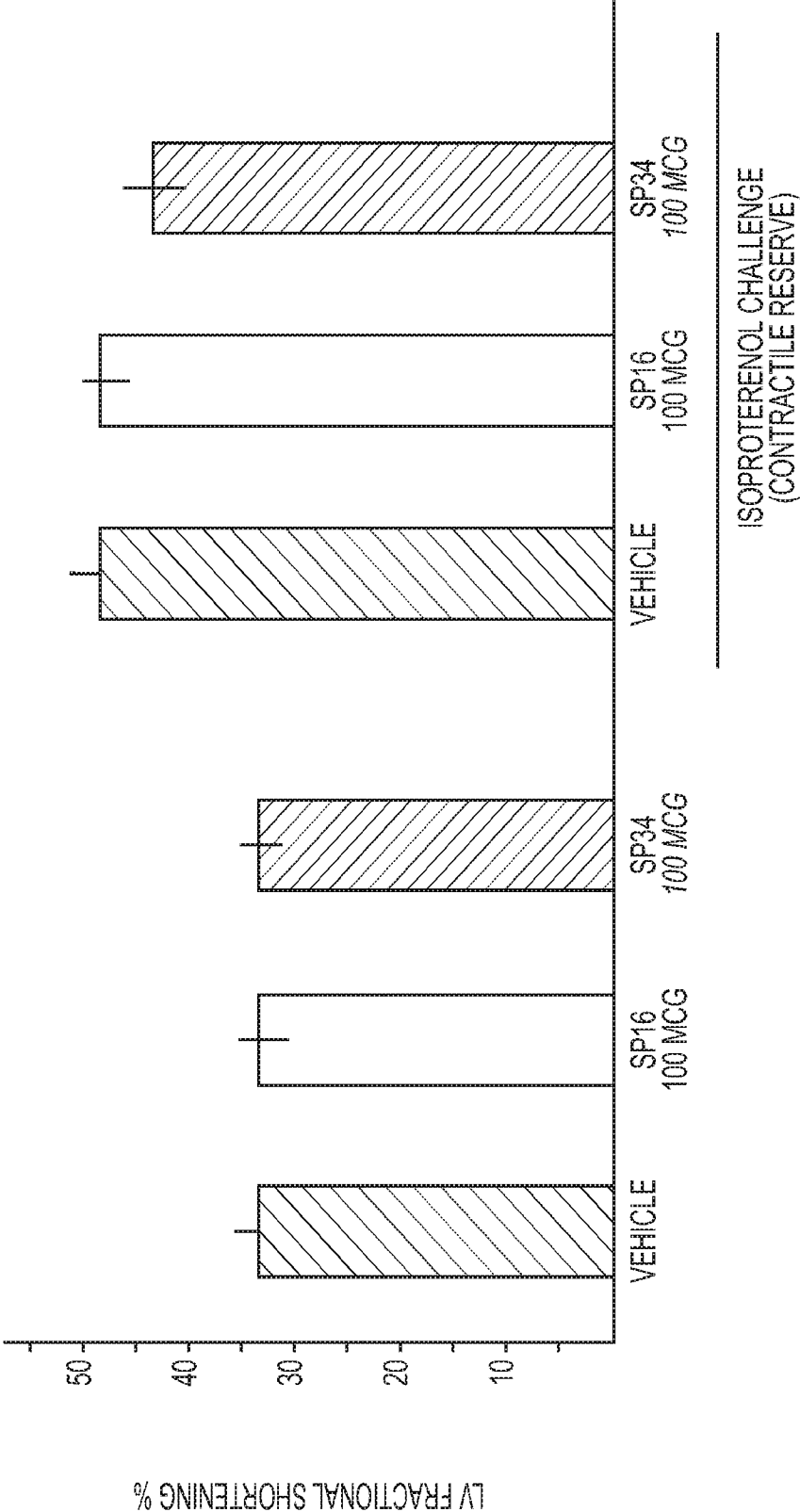


FIG. 7

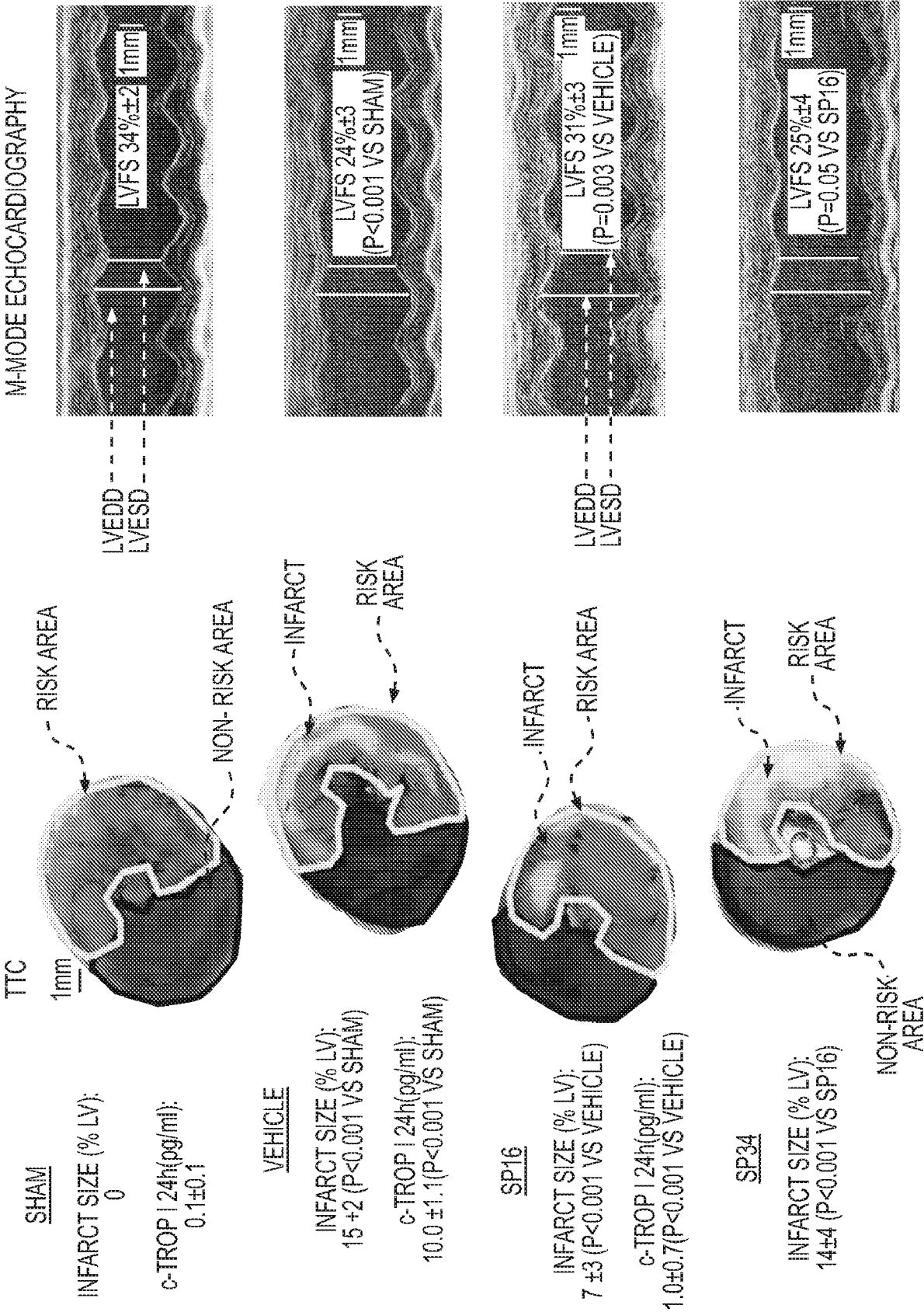
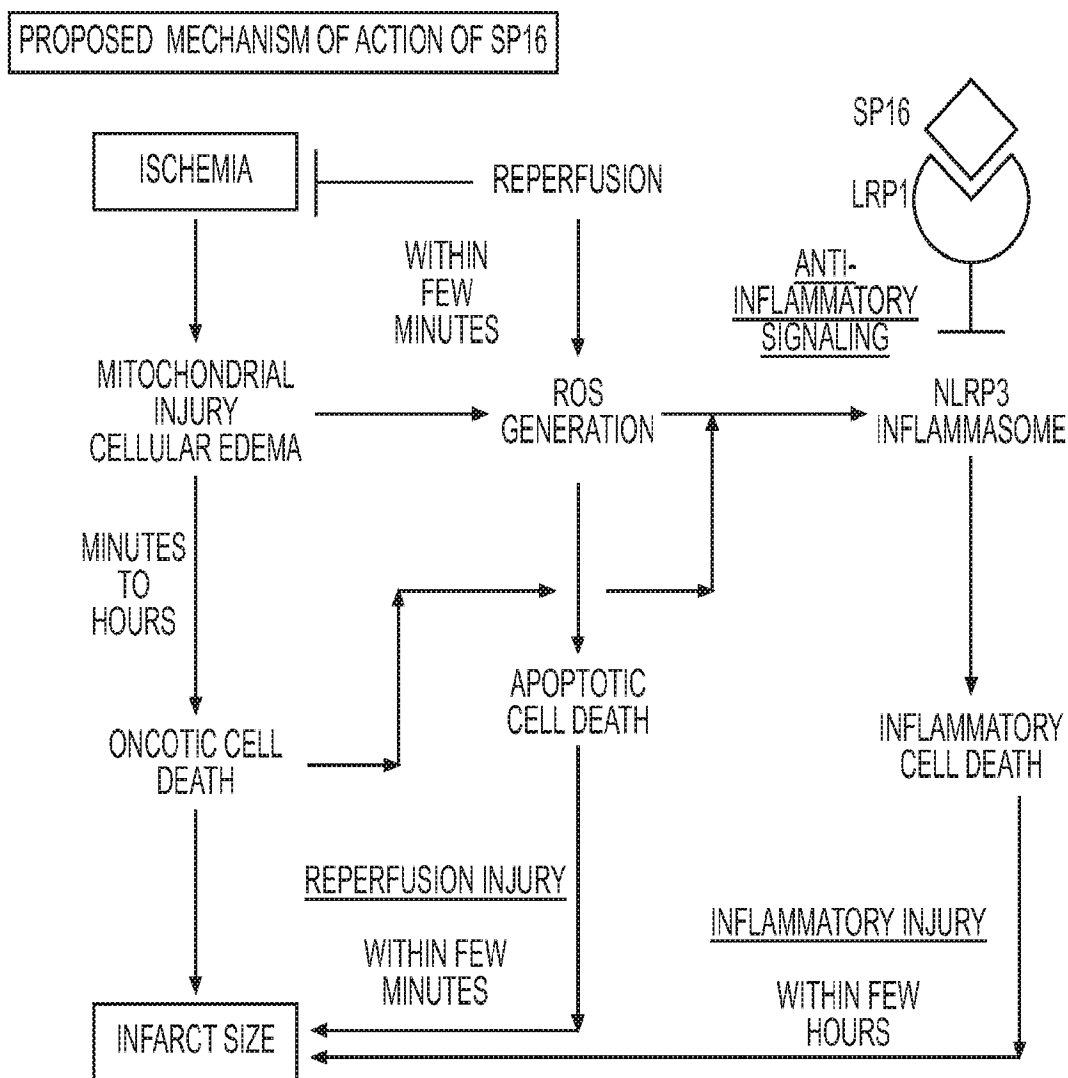


FIG. 8

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**FIG. 9**

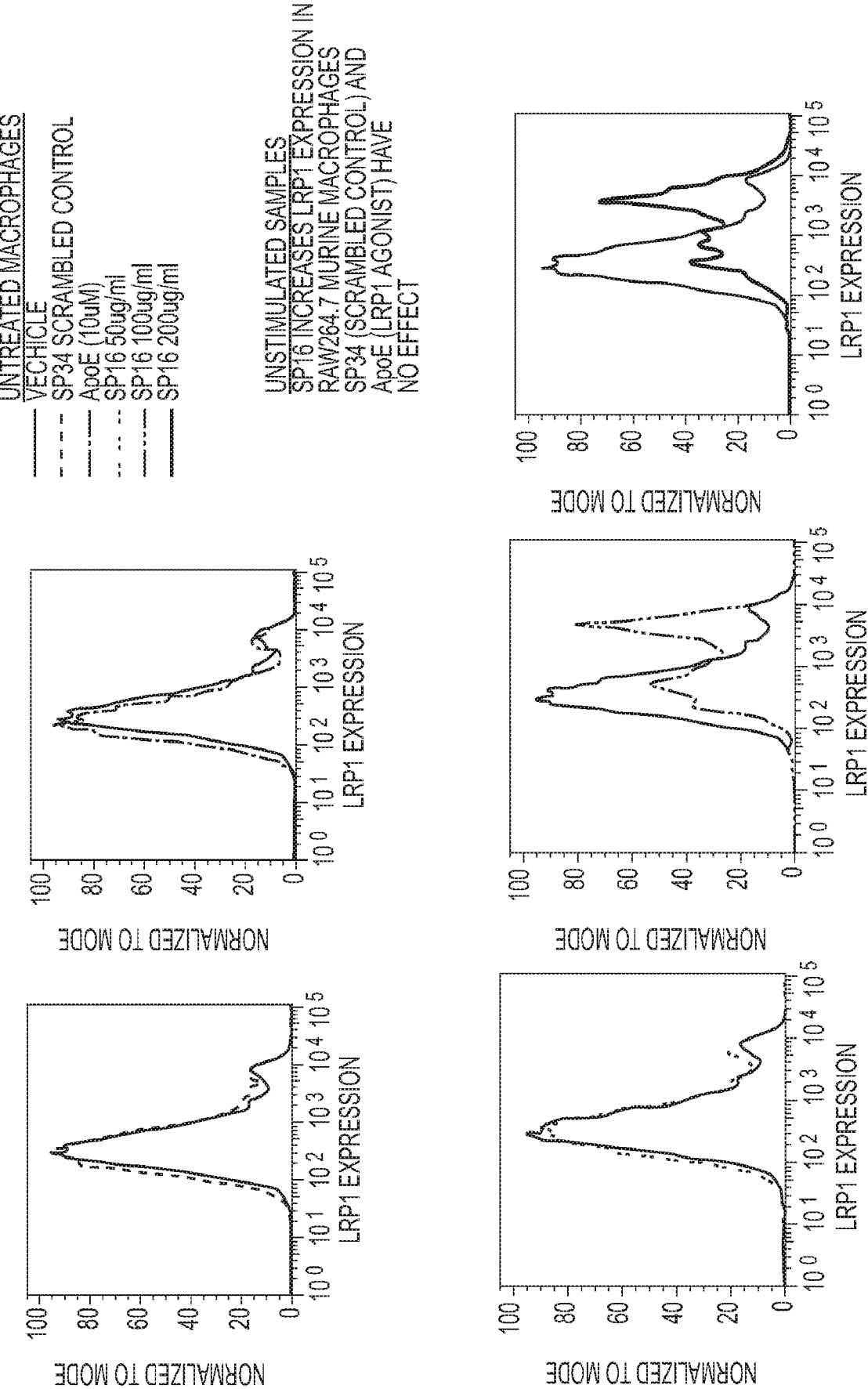


FIG. 10

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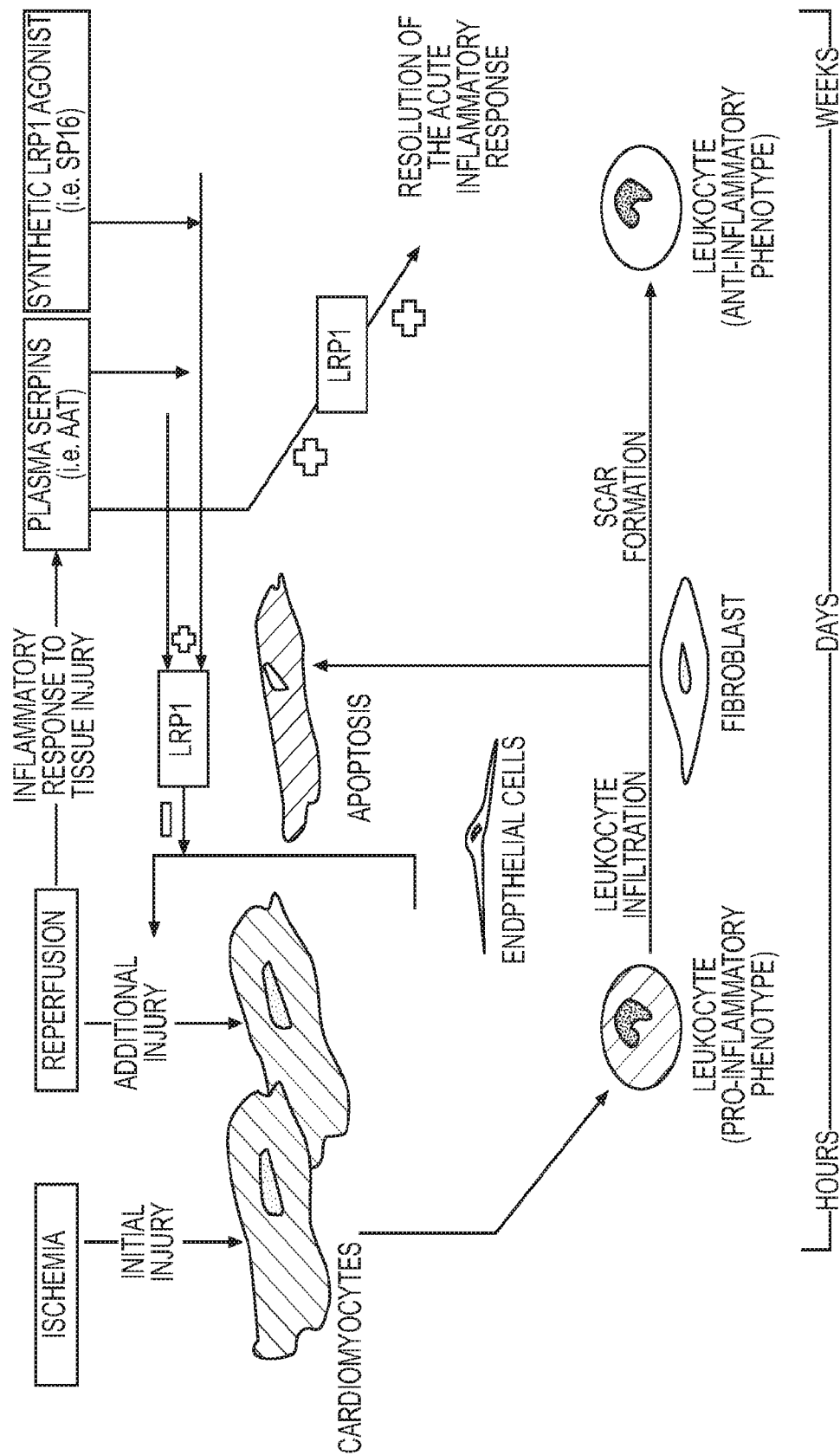
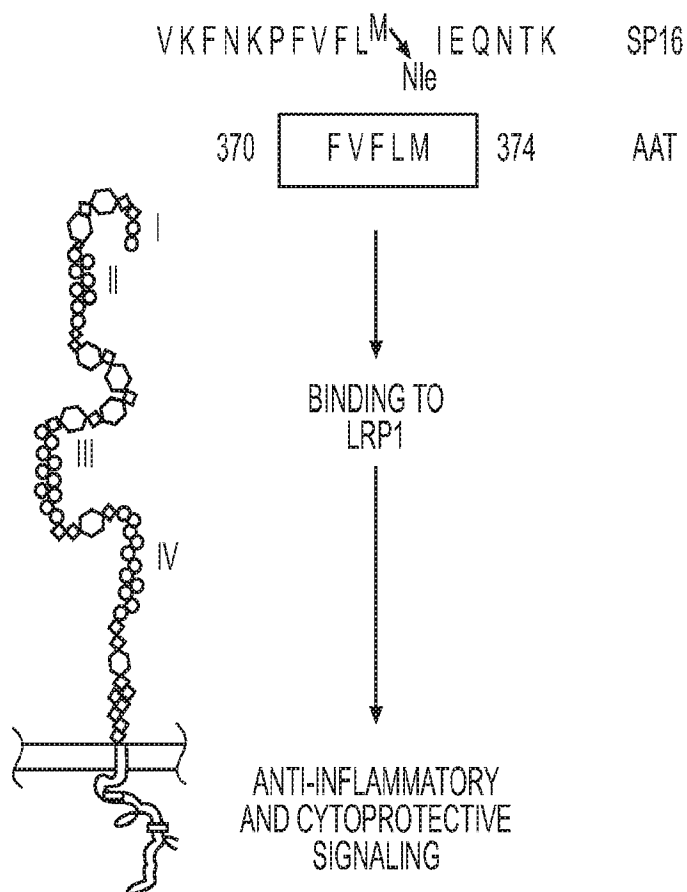
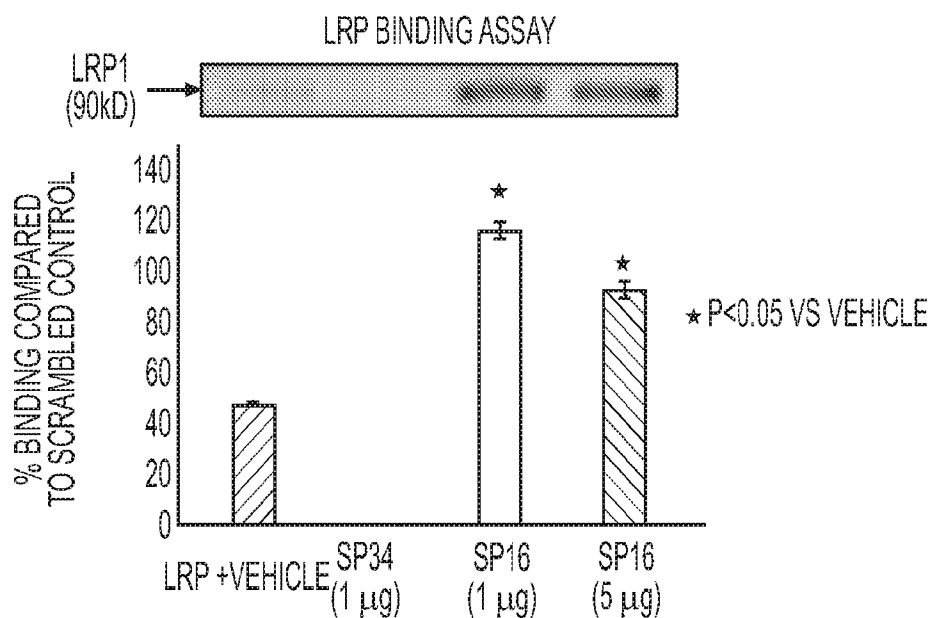
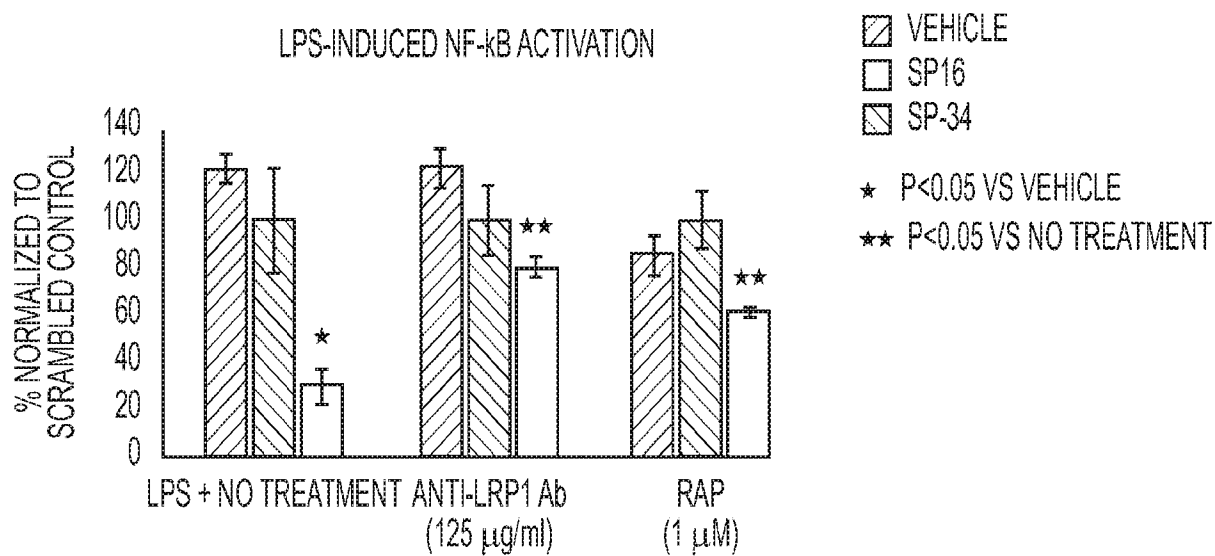
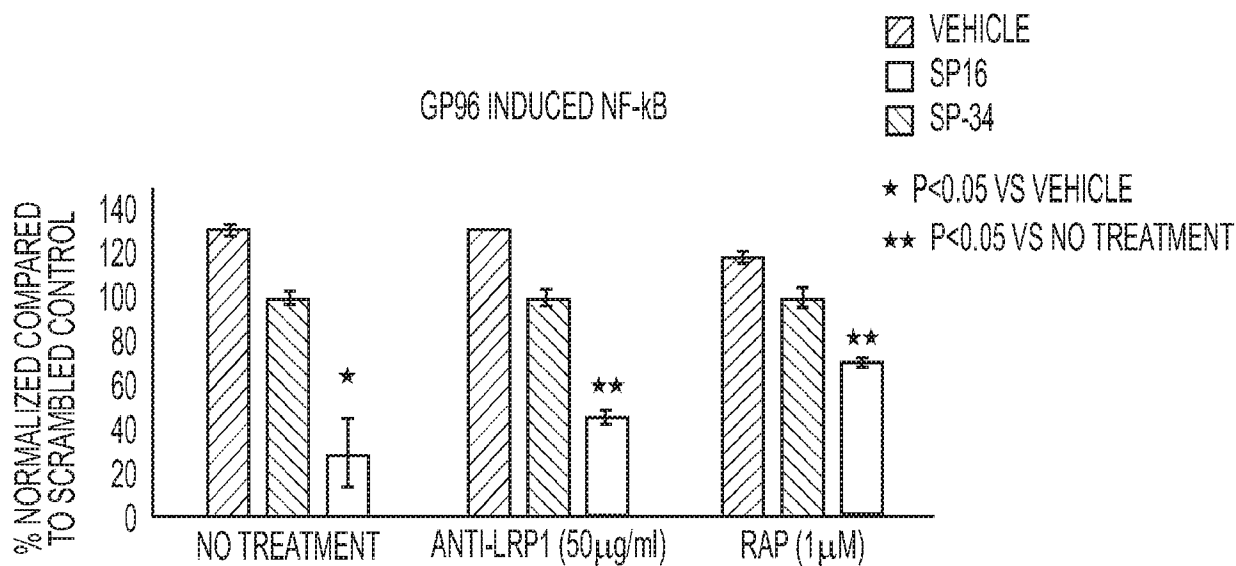


FIG. 11

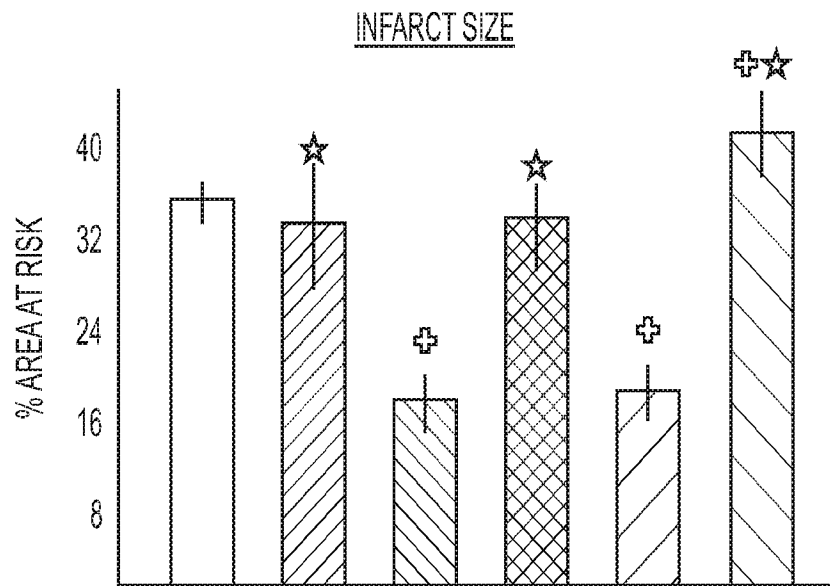
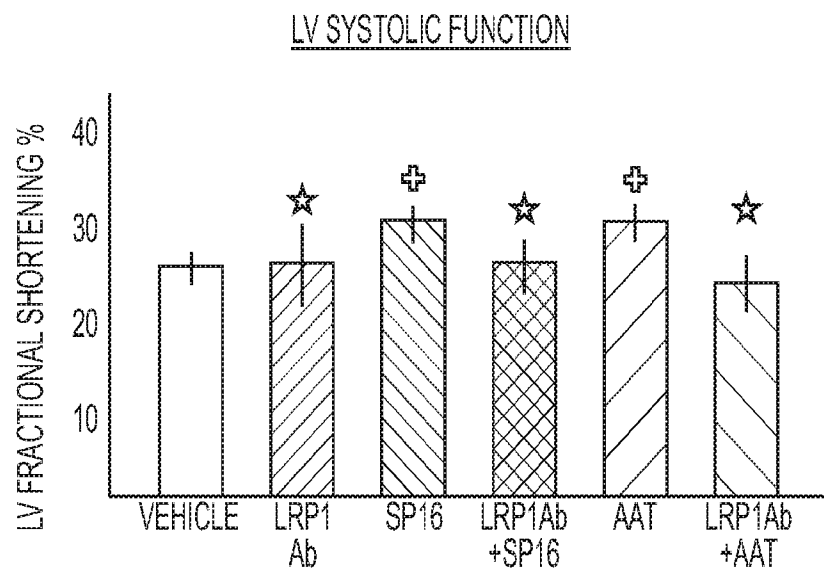
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**FIG. 12A****FIG. 12B**

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**FIG. 13A****FIG. 13B**

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**FIG. 14A**

ISCHEMIA(30min)-REPERFUSION(24h)

+ P<0.01 VS VEHICLE; * P<0.05 VS SP16

FIG. 14B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/048999

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/201; A61K 31/365; A61K 31/436 (2016.01)

CPC - A61K 35/17; C12N 9/12; C12N 9/90 (2016.11)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC - A61K 31/201; A61K 31/365; A61K 31/436

CPC - A61K 35/17; C12N 9/12; C12N 9/90

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 424/93.21; 435/188; 435/325 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Patbase, Google Patents, PubMed

Search terms used: SP16 cytokine storm hypercytokinemia administer oral AML acute myocardial infarction cardiac human

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2013/0274187 A1 (SERPIN PHARMA LLC) 17 October 2013 (17.10.2013) entire document	1, 6
--		-----
Y		2-5
Y	US 2009/0023643 A1 (SOUTHARD et al) 22 January 2009 (22.01.2009) entire document	2-5
A	US 8,288,350 B2 (ELGEBALY et al) 16 October 2012 (16.10.2012) entire document	1-6
A	US 2009/0005348 A1 (OGRU et al) 01 January 2009 (01.01.2009) entire document	1-6
A	US 2013/0071389 A1 (TRACEY et al) 21 March 2013 (21.03.2013) entire document	1-6

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

21 December 2016

Date of mailing of the international search report

05 JAN 2017

Name and mailing address of the ISA/US

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Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/048999

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☒ forming part of the international application as filed:

☒ in the form of an Annex C/ST.25 text file.

☒ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NO:1 was searched.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/048999

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 7-16
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see Extra Sheet(s).

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-6 to the extent that they read on a peptide of SEQ ID NO:1.

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Continued from Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-6 are drawn to peptides, and methods of treating diseases comprising the same.

The first invention of Group I+ is restricted to a peptide, and methods of treating diseases comprising the same, wherein the peptide is selected to be SEQ ID NO: 1. It is believed that claims 1-6 read on this first named invention and thus these claims will be searched without fee to the extent that they read on a peptide of SEQ ID NO:1.

Applicant is invited to elect additional peptides, each with specified SEQ ID NO, to be searched in a specific combination by paying an additional fee for each set of election. An exemplary election would be a peptide, and methods of treating diseases comprising the same, wherein the peptide is selected to be SEQ ID NO:4. Additional peptides, each with specified SEQ ID NO, will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulas do not share a significant structural element responsible for treating acute myocardial infarction (AMI) and/or disease associated with a cytokine storm, requiring the selection of alternatives for the therapeutic peptide, where "a peptide selected from the group consisting of: (i) a peptide comprising the amino acid sequence X1-Z1-F-N-K-P-F-X2-Z2-X3-Z3-Q (SEQ ID NO: 2), wherein X1 is V or L; X2 is V, L, Nle, or M; X3 is M, Nle, I or V; Z1 is any amino acid; Z2 is a sequence of any two amino acids; and Z3 is a sequence any five amino acids, and wherein the peptide comprises or fewer amino acids; (j) a peptide comprising the amino acid sequence VKFNKPFVFLMIEQNTK (SEQ ID NO: 1); (k) a peptide consisting essentially of the amino acid sequence X1-Z1-F-N-X2-P-F-X3-Z2-X4-Z3-X5 (SEQ ID NO: 3), wherein X1 is V or L; X2 is K or R; X3 is V, L, Nle, or M; X4 is M, Nle, I or V; X5 is K or Q; Z1 is any amino acid; Z2 is a sequence of any two amino acids; and Z3 is a sequence any five amino acids; (l) a peptide consisting essentially of the amino acid sequence RFNRPFLR (SEQ ID NO: 4); (m) a peptide consisting essentially of the amino acid sequence of RRRFNRPFLRRR (SEQ ID NO: 8); (n) a peptide consisting essentially of the amino acid sequence of VKFNKPFVFLMIEQNTK (SEQ ID NO: 1); (o) a peptide consisting essentially of the amino acid sequence of FNRPF (SEQ ID NO: 10); and (p) a peptide comprising SEQ ID NO: 57".

The Groups I+ share the technical features of a method of treating a disease associated with a cytokine storm, comprising administering to a human subject affected with the disease a pharmaceutical composition comprising a peptide; a method of reducing infarct size in a human subject in need of treatment therefor, comprising administering to the human subject a pharmaceutical composition comprising a peptide; a method of treating acute myocardial infarction (AMI), comprising administering to a human subject affected with AMI a pharmaceutical composition comprising a peptide. However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 20090023643 A1 to Southard et al. discloses a method of treating a disease associated with a cytokine storm (This invention relates to methods of treating acute myocardial infarction by administering calcitonin gene related peptide, Abstract; CGRP may be acting as a cardioprotective agent based on its ability to modulate cytokines during inflammation associated with AMI, Para. [0043]) comprising administering to a human subject affected with the disease a pharmaceutical composition comprising a peptide (administering CGRP to a subject in need of such treatment and compositions, Para. [0010]; treating a subject suspected of having a myocardial infarction or preventing an acute myocardial infarction in a subject, comprising administering CGRP to a subject in need of such treatment, Para. [0011]; human subject, Para. [0027]); a method of reducing infarct size in a human subject in need of treatment therefor, (ameliorating one or more symptoms associated with acute myocardial infarction, cardioprotection, reduction in infarction size, Para. [0028]; treating a subject suspected of having a myocardial infarction or preventing an acute myocardial infarction in a subject, comprising administering CGRP to a subject in need of such treatment, Para. [0011]) comprising administering to the human subject a pharmaceutical composition comprising a peptide (administering CGRP to a subject in need of such treatment and compositions, Para. [0010]; treating a subject suspected of having a myocardial infarction or preventing an acute myocardial infarction in a subject, comprising administering CGRP to a subject in need of such treatment, Para. [0011]; human subject, Para. [0027]); a method of treating acute myocardial infarction (AMI) (This invention relates to methods of treating acute myocardial infarction by administering calcitonin gene related peptide, Abstract; CGRP may be acting as a cardioprotective agent based on its ability to modulate cytokines during inflammation associated with AMI, Para. [0043]), comprising administering to a human subject affected with AMI a pharmaceutical composition comprising a peptide (administering CGRP to a subject in need of such treatment and compositions, Para. [0010]; treating a subject suspected of having a myocardial infarction or preventing an acute myocardial infarction in a subject, comprising administering CGRP to a subject in need of such treatment, Para. [0011]; human subject, Para. [0027]).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.