catastrophic; a major disability (including limb loss) and even death can result. Foot ulceration is the precursor to about 85% of lower extremity amputations in persons with diabetes (s*ee, e.g.,* Apelqvist, et al., What is the most effective way to reduce incidence of amputation in the diabetic foot? *Diabetes Metab Res. Rev.,* 16(1 Suppl.): S75-S83 (2000)). Thus, there is a need for accelerating or improving wound healing, including diabetic wound healing.

SUMMARY

In one example, the disclosure provides IL-22 Fc fusion proteins, compositions comprising the same, and methods of using the same.

In one example, the disclosure provides an IL-22 Fc fusion protein that binds to IL-22 receptor, said IL-22 Fc fusion protein comprising an IL-22 polypeptide linked to an Fc region by a linker, wherein the Fc region comprises a hinge region, an IgG CH2 domain and an IgG CH3 domain, wherein the IL-22 Fc fusion protein comprises an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, preferably at least 99% sequence identity to the amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 and SEQ ID NO:14, and wherein the Fc region is not glycosylated. In certain examples, the N297 residue of the CH2 domain is changed to glycine or alanine. In certain other examples, the N297 residue is changed to Gly; while in other examples, the N297 residue is changed to Ala. In certain examples, the binding to IL-22 receptor triggers IL-22 receptor downstream signaling, including activating STAT3.

In one aspect, the present disclosure refers to an IL-22 Fc fusion protein comprising an IL-22 polypeptide linked to an IgG Fc region by a linker, wherein the Fc region comprises an IgG1 Fc region or an IgG4 Fc region, and wherein the Fc region is not glycosylated.

In certain examples, the IL-22 Fc fusion protein comprises an amino acid sequence having at least 98% sequence identity to the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:12. In certain other examples, the IL-22 Fc fusion protein comprises an amino acid sequence having at least 99% sequence identity to the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:12. In certain other examples, the IL-22 Fc fusion protein comprises an amino acid sequence having at least 99% sequence identity to the amino acid sequence of SEQ ID NO:8. In certain other examples, the IL-22 Fc fusion protein comprises an amino acid sequence having at least 99% sequence identity to the amino acid sequence of SEQ ID NO:12. In certain examples, the functions and/or activities of the IL-22 Fc fusion protein can be assayed by *in vitro* or *in vivo* methods, for example, IL-22 receptor binding assay, Stat3 luciferase reporter activity assay, etc. In certain examples, the IL-22 Fc fusion protein comprises the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:12. In certain particular examples, the IL-22 Fc fusion protein comprises the amino acid sequence of SEQ ID NO:8. In certain examples, the disclosure provides the IL-22 Fc fusion protein produced by the

method comprising the step of culturing a host cell capable of expressing the IL-22 Fc fusion protein under conditions suitable for expression of the IL-22 Fc fusion protein. In certain examples, the method further comprises the step of obtaining the IL-22 Fc fusion protein from the cell culture or culture medium. In certain examples, the host cell is a Chinese hamster ovary (CHO) cell; while in other examples, the host cell is an *E. coli* cell.

In another aspect, the present disclosure refers to an IL-22 Fc fusion protein comprising the amino acid sequence of SEQ ID NO:12.

In yet another aspect, the present disclosure refers to an IL-22 Fc fusion protein comprising the amino acid sequence of SEQ ID NO:14.

In another aspect, the present disclosure refers to an IL-22 Fc fusion protein comprising the amino acid sequence of SEQ ID NO:20.

In another aspect, the present disclosure refers to an IL-22 Fc fusion protein consisting of the amino acid sequence of SEQ ID NO:12.

In yet another aspect, the present disclosure refers to an IL-22 Fc fusion protein consisting of the amino acid sequence of SEQ ID NO:14.

In another aspect, the present disclosure refers to an IL-22 Fc fusion protein consisting of the amino acid sequence of SEQ ID NO:20.

In another example, the disclosure provides an IL-22 Fc fusion protein comprising an IL-22 polypeptide linked to an IgG Fc region by a linker, wherein the Fc region comprises a hinge region, an IgG CH2 domain and an IgG CH3 domain, and wherein the Fc region is not glycosylated. In certain examples, the hinge region comprises the amino acid sequence of CPPCP (SEQ ID NO:31). In certain other examples, the N297 residue in the Fc region is changed and/or the T299 residue in the Fc region is changed. In certain examples, the N297 residue in the CH2 domain is changed, preferably to glycine or alanine. In certain particular examples, the N297 residue is changed to glycine. In certain other examples, the N297 residue is changed to alanine. In yet other examples, the T299 residue is changed to Ala, Gly or Val. In certain other examples, the linker is 8-20 amino acids long, 8-16 amino acids long, or 10-16 amino acids long.

In certain examples, the Fc region comprises the CH2 and CH3 domain of IgG1. In certain particular examples, the linker comprises the amino acid sequence DKTHT (SEQ ID NO:32). In certain examples, the linker comprises the amino acid sequence GGGDKTHT (SEQ ID NO:41). In certain examples, the linker is at least 11 amino acids long and comprises the amino acid sequence EPKSCDKTHT (SEQ ID NO:33). In certain other examples, the linker comprises the amino acid sequence VEPKSCDKTHT (SEQ ID NO:34), KVEPKSCDKTHT (SEQ ID NO:35), KKVEPKSCDKTHT (SEQ ID NO:36), DKKVEPKSCDKTHT (SEQ ID NO:37), VDKKVEPKSCDKTHT (SEQ ID NO:38), or KVDKKVEPKSCDKTHT (SEQ ID NO:39). In certain particular examples, the linker comprises the amino acid sequence EPKSSDKTHT (SEQ ID NO:40). In certain examples, the linker comprises the amino acid sequence VEPKSSDKTHT (SEQ ID NO:67), KVEPKSSDKTHT (SEQ ID NO:68), KKVEPKSSDKTHT (SEQ ID NO:66), DKKVEPKSSDKTHT (SEQ ID NO:64), VDKKVEPKSSDKTHT (SEQ ID NO:69), or KVDKKVEPKSSDKTHT (SEQ ID NO:65). In certain particular examples, the linker does not comprise the amino acid sequence of GGS (SEQ ID NO: 45), GGGS (SEQ ID NO:46) or GGGGS (SEQ ID NO:47). In separate examples, the IL-22 IgG1 Fc fusion protein comprises a linker sequence of GGGSTHT (SEQ ID NO:63). In other particular examples, the IL-22 Fc fusion protein comprises the amino acid sequence of SEQ ID NO:12 or SEQ ID

NO:14. In certain other particular examples, the IL-22 Fc fusion protein comprises the amino acid sequence of SEQ ID NO:12.

In certain examples, the IL-22 Fc fusion protein comprises the CH2 and CH3 domain of IgG4. In certain other examples, the linker comprises the amino acid sequence SKYGPP (SEQ ID NO:43). In certain particular examples, the linker comprises the amino acid sequence RVESKYGPP (SEQ ID NO:44). In certain examples, none of the linkers comprise the amino acid sequence GGS (SEQ ID NO:45), GGGS (SEQ ID NO:46) or GGGGS (SEQ ID NO:47). In other particular examples, the IL-22 Fc fusion protein comprises the amino acid sequence of SEQ ID NO:8 or SE ID NO:10. In particular examples, the IL-22 Fc fusion protein comprises the amino acid sequence of SEQ ID NO:8. In another example, the IL-22 Fc fusion protein is produced by the method comprising the step of culturing a host cell capable of expressing the IL-22 Fc fusion protein under conditions suitable for expression of the IL-22 Fc fusion protein. In certain examples, the IL-22 Fc fusion protein is produced by the method that further comprises the step of obtaining the IL-22 Fc fusion protein from the cell culture or culture medium. In certain examples, the host cell is a Chinese hamster ovary (CHO) cell. In certain other examples, the host cell is an *E. coli* cell.

In yet another example, the disclosure provides a composition comprising an IL-22 Fc fusion protein, said IL-22 Fc fusion protein comprising an IL-22 polypeptide linked to an Fc region by a linker, wherein the Fc region comprises a hinge region, an IgG CH2 domain and an IgG CH3 domain, and wherein the composition has an afucosylation level in the CH2 domain of no more than 5%. In certain examples, the afucosylation level is no more than 2%, more preferably less than 1%. In certain examples, the afucosylation level is measured by mass spectrometry. In certain examples, the Fc region comprises the CH2 and CH3 domain of IgG4. In certain examples, the Fc region comprises a CH2 and CH3 domain of IgG1. In certain other examples, the hinge region comprises the amino acid sequence of CPPCP (SEQ ID NO:31). In certain examples, the IL-22 Fc fusion protein comprises the amino acid sequence of SEQ ID NO:24 or SEQ ID NO:26. In certain examples, the IL-22 Fc fusion protein comprises the amino acid sequence of SEQ ID NO:24. In certain examples, the composition is produced by the process comprising the steps of culturing a host cell capable of expressing the IL-22 Fc fusion protein under conditions suitable for expression of the IL-22 Fc fusion protein, and obtaining the IL-22 Fc fusion protein from the cell culture or culture medium, wherein the composition has an afucosylation level in the CH2 domain of the Fc region of no more than 5%. In certain examples, the afucosylation level is no more than 2%, more preferably less than 1%. In certain examples, the IL-22 Fc fusion protein is

obtained by purification, preferably purifying fucosylated species away from afucosylated species. In certain examples, the IL-22 Fc fusion protein is purified by affinity chromatography. In certain examples, the host cell is a CHO cell.

In a further example, the disclosure provides an IL-22 Fc fusion protein, or a composition comprising IL-22 Fc fusion proteins, said IL-22 Fc fusion protein is produced by the process comprising the step of culturing a host cell capable of expressing the IL-22 Fc fusion protein under conditions suitable for expression of the IL-22 Fc fusion protein. In certain examples, the process further comprises the step of obtaining the IL-22 Fc fusion protein from the cell culture or culture medium. In certain examples, the host cell is a CHO cell; while in other examples, the host cell is an *E. coli* cell.

In a further example, the disclosure provides a composition comprising an IL-22 Fc fusion protein described herein. In yet another example, the disclosure provides a pharmaceutical composition comprising an IL-22 Fc fusion protein described herein, and at least one pharmaceutically acceptable carrier. In certain examples, the composition or pharmaceutical composition comprises an IL-22 Fc fusion protein comprising an amino acid sequence of SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:24 or SEQ ID NO:26. In certain particular examples, the composition or pharmaceutical composition comprises an IL-22 Fc fusion protein comprising the amino acid sequence of SEQ ID NO:8. In certain particular examples, the IL-22 Fc fusion protein is produced by *E. coli*. In certain other examples, the Fc region of the IL-22 Fc fusion protein is not glycosylated. In certain further examples, the IL-22 Fc fusion protein does not induce antibody dependent cellular cytotoxicity (ADCC). In certain examples, the pharmaceutical composition further comprises a suboptimal amount of a therapeutic agent such as dexamethasone. In certain examples, the IL-22 polypeptide comprises the amino acid sequence of SEQ ID NO:4.

Further, according to each and every example of the disclosure, in certain examples, the IL-22 Fc fusion protein can be a dimeric IL-22 Fc fusion protein (with respect to IL-22); while in other examples, the IL-22 Fc fusion protein can be a monomeric Fc fusion protein (with respect to IL22).

In a further example, the disclosure provides a monomeric IL-22 Fc fusion protein. In certain particular examples, the monomeric fusion protein comprises an IL-22 Fc fusion arm and an Fc arm. In certain examples, the IL-22 Fc fusion arm and the Fc arm comprises either a knob or a hole in the Fc region. In certain examples, the Fc region of the IL-22 Fc fusion arm (the monomer IL-22 Fc fusion) comprises a knob and the Fc region of the Fc arm (the monomer Fc without linking to IL-22) comprises a hole. In certain examples, the Fc

region of the IL-22 Fc fusion arm (the monomer IL-22 Fc fusion) comprises a hole and the Fc region of the Fc arm (the monomer Fc without linking to IL-22) comprises a knob. In certain other examples, the monomeric IL-22 Fc fusion protein comprises the amino acid sequence of SEQ ID NO:61 and SEQ ID NO:62. In certain other examples, the Fc region of both arms further comprises an N297G mutation. In certain examples, the monomeric IL-22 Fc is produced by the process comprising the step of culturing one or more host cells comprising one or more nucleic acid molecules capable of expressing the first polypeptide comprising the amino acid sequence of SEQ ID NO:61 and the second polypeptide comprising the amino acid sequence of SEQ ID NO:62. In certain other examples, the method further comprises the step of obtaining the monomeric IL-22 Fc fusion protein from the cell culture or culture medium. In certain examples, the host cell is an E. coli cell. In a related example, the disclosure provides a composition or pharmaceutical composition comprising the monomeric IL-22 Fc fusion protein.

In yet another aspect, the present disclosure refers to an isolated nucleic acid encoding the IL-22 Fc fusion protein discosed herein. In certain examples, the nucleic acid encodes the IL-22 Fc fusion protein comprising the amino acid sequence of SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:24 or SEQ ID NO:26, preferably SEQ ID NO:8 or SEQ ID NO:12, more preferably SEQ ID NO:8. In certain other examples, the nucleic acid comprises the polynucleotide sequence of SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:23 or SEQ ID NO:25. In certain particular examples, the nucleic acid comprises the polynucleotide sequence of SEQ ID NO:7 or SEQ ID NO:11, preferably SEQ ID NO:7. In certain examples, the isolated nucleic acid comprises a polynucleotide sequence that is at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the polynucleotide sequence of SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13; SEQ ID NO:23 or SEQ ID NO:25. In certain examples, the isolated nucleic acid comprises a polynucleotide sequence that is at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the polynucleotide sequence of SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13; SEQ ID NO:23 or SEQ ID NO:25, wherein the isolated nucleic acid is capable of encoding an IL-22 Fc fusion protein that is capable of binding to IL-22R and/or triggering IL-22R activity and wherein the Fc region of the IL-22 Fc fusion protein is not glycosylated. In certain examples, the isolated nucleic acid comprises a polynucleotide sequence that is at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the polynucleotide

sequence of SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13; SEQ ID NO:23 or SEQ ID NO:25, wherein the isolated nucleic acid is capable of encoding an IL-22 Fc fusion protein comprising the amino acid sequence of SEQ ID NO:8, 10, 12, or 14. In related examples, the disclosure provides vectors comprising the nucleic acid described above, and a host cell comprising the vector. In certain examples, the host cell is a prokaryotic cell or eukaryotic cell. In certain particular examples, the host cell is a prokaryotic cell, including without limitation, an *E. coli* cell. In certain other examples, the host cell is a eukaryotic cell, including without limitation, a CHO cell. In certain examples, the host cell comprises a vector comprising a nucleic acid encoding the IL-22 Fc fusion protein comprising the amino acid sequence of SEQ ID NO:8.

In one aspect, the present disclosure refers to a vector comprising the nucleic acid disclosed herein.

In another aspect, the present disclosure refers to a host cell comprising the vector disclosed herein.

In a further related aspect, the present disclosure refers to a method of making the IL-22 Fc fusion protein comprising the step of culturing the host cell disclosed herein under conditions suitable for expression of the IL-22 Fc fusion protein. In certain examples, the method further comprises the step of obtaining the IL-22 Fc fusion protein from the cell culture or culture medium. The IL-22 Fc fusion protein can be obtained from the cell culture or culture medium by any methods of protein isolation or purification known in the art, including without limitation, collecting culture medium, freezing/thawing, centrifugation, cell lysis, homogenization, ammonium sulfate precipitation, HPLC, and affinity, gel filtration, and ion exchanger column chromatography. In certain examples, the method further comprises the step of removing afucosylated IL-22 Fc fusion protein. In certain other examples, the afucosylated IL-22 Fc fusion protein is removed by affinity column chromatography. In certain examples, the host cell is an *E. coli* cell. In other examples, the host cell is a CHO cell.

In yet another example, the disclosure provides a composition or pharmaceutical composition comprising an IL-22 Fc fusion protein of the disclosure and at least one pharmaceutically acceptable carrier. In certain examples, the IL-22 Fc fusion protein comprises the amino acid sequence of SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:24, or SEQ ID NO:26. In other examples, the Fc region of the IL-22 Fc fusion protein is not glycosylated. In certain examples, the Fc region of the IL-22 Fc fusion protein is not glycosylated while the IL-22 polypeptide is glycosylated. In certan such examples, the IL-22 Fc fusion protein is produced in CHO cells. In certain examples, the IL-22 Fc fusion protein does not induce antibody dependent cellular cytotoxicity. In yet other examples, the pharmaceutical composition further comprises dexamethasone or a TNF antagonist. In certain particular examples, the dexamethasone or a TNF antagonist is present at a suboptimal amount.

In one aspect, the present disclosure refers to a pharmaceutical composition comprising the IL-22 Fc fusion protein disclosed herein and at least one pharmaceutically acceptable carrier.

In another aspect, the present disclosure refers to a pharmaceutical composition comprising an IL-22 polypeptide or an IL-22 Fc fusion protein disclosed herein and at least one pharmaceutically acceptable carrier, wherein the pharmaceutically acceptable carrier is a gelling agent.

In certain other examples, the pharmaceutical composition comprising IL-22 Fc fusion proteins has an afucosylation level in the CH2 domain of no more than 5%, preferably no more than 2%, more preferably less than 1%. In certain particular examples, the IL-22 Fc fusion protein comprises the amino acid sequence of SEQ ID NO:24 or SEQ ID NO:26, preferably SEQ ID NO:24. In certain other examples, the IL-22 Fc fusion protein is produced in CHO cells. In certain particular examples, the subject is a human. In certain examples, the pharmaceutical composition is administered systematically or topically. In certain other examples, the pharmaceutical composition is administered intravenously, subcutaneously, intraperitoneally or topically.

In a further example, the disclosure provides a pharmaceutical composition comprising an IL-22 polypeptide or IL-22 Fc fusion protein described herein and at least one pharmaceutically acceptable carrier. In certain examples, the pharmaceutically acceptable carrier is a gelling agent. In certain examples, the gelling agent is a polysaccharide. In some examples, the gelling agent is, without limitation, methylcellulose, hydroxyethyl cellulose, carboxymethyl cellulose, hydroxypropyl cellulose, POE-POP block polymers, alginate, hyaluronic acid, polyacrylic acid, hydroxyethyl methylcellulose or hydroxypropyl methylcellulose. In some examples, the polysaccharide is a cellulosic agent such as, without limitation, hydroxyethyl methylcellulose or hydroxypropyl methylcellulose. In certain examples, the gelling agent is hydroxypropyl methylcellulose. In some examples, the pharmaceutical composition is for topical administration. In certain examples, the pharmaceutical composition for topical administration comprises an IL-22 polypeptide. In some examples, the pharmaceutical composition for topical administration comprises an IL-22 Fc fusion protein. In certain examples, the pharmaceutical composition for topical administration comprises an IL-22 polypeptide without an Fc fusion.

In one aspect, the present disclosure refers to the use of the IL-22 Fc fusion protein disclosed herein or the pharmaceutical composition disclosed herein in the manufacture of a medicament for treating inflammatory bowel disease (IBD) in a subject in need thereof.

In another example, the disclosure provides methods of treating IBD in a subject in need thereof comprising administering to the subject the pharmaceutical composition comprising an IL-22 Fc fusion protein of the disclosure. In certain examples, the IBD is ulcerative colitis. In certain other examples, the IBD is Crohn's disease. In certain particular examples, the Fc region of the IL-22 Fc fusion protein is not glycosylated. In certain examples, the N297 residue and/or the T299 residue of the Fc region is changed. In certain examples, the N297 residue of the Fc region is changed. In certain other examples, the N297 residue is changed to Gly or Ala, preferably Gly. In certain other examples, the T299 residue is changed, preferably to Val, Gly or Ala. In certain particular examples, the IL-22 Fc fusion protein comprises the amino acid sequence of SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or

SEQ ID NO:14, preferably SEQ ID NO:8. In certain examples, the IL-22 Fc fusion protein is produced in E. coli or a CHO cell. In certain examples, the subject is a human. In certain other examples, the pharmaceutical composition is administered intravenously, subcutaneously, intraperitoneally or topically.

In another example, the disclosure provides methods of treating any one or combination of the following diseases using an IL-22 polypeptide or an IL-22 Fc fusion protein of this disclosure: Type II diabetes, Type II diabetes with morbid obesity, wounds (including diabetic wounds and diabetic ulcers), burns, ulcers (including pressure ulcer and venous ulcer), graft versus host disease (GVHD), atherosclerosis, cardiovascular disease, metabolic syndrome, endotoxemia (acute and mild), sepsis, acute coronary heart disease, hypertension, dyslipemia, obesity, hyperglycemia, lipid metabolism disorders, hepatitis, acute hepatitis, renal failure, acute renal failure, acute kidney injury, renal draft failure, post cadaveric renal transplant delayed graft function, contrast induced nephropathy, pancreatitis, acute pancreatitis, liver fibrosis and lung fibrosis. In certain examples, acute pancreatitis can be mild to moderate to severe disease. In certain examples, acute pancreatitis includes disease post ERCP (endoscopic retrograde cholangiopancreatography). In some further examples, the patient to be treated for the above disease is in need of a change in his HDL/LDL lipid profile, which IL-22 polypeptide or IL-22 Fc fusion proteins can alter in the patient to increase HDL and decrease LDL. In a related example, the disclosure provides uses of an IL-22 polypeptide or an IL-22 Fc fusion protein in the preparation of a medicament for the treatment of any one or combinations of the above diseases.

In a further example, the disclosure provides methods of inhibiting microbial infection in the intestine, or preserving goblet cells in the intestine during a microbial infection, of a subject in need thereof comprising the step of administering to the subject the pharmaceutical composition comprising the IL-22 Fc fusion protein of the disclosure. In other related examples, the disclosure provides methods of enhancing epithelial cell integrity, mucosal healing, epithelial cell proliferation, epithelial cell differentiation, epithelial cell migration or epithelial wound healing in the intestine in a subject in need thereof comprising administering to the subject the pharmaceutical composition comprising the IL-22 Fc fusion protein of the disclosure. In certain examples, the epithelial cell is intestinal epithelial cell.

In one aspect, the present disclosure refers to the use of the IL-22 Fc fusion protein disclosed herein or the pharmaceutical composition disclosed herein in the manufacture of a medicament for inhibiting microbial infection in the intestine, preserving goblet cells in the intestine during a microbial infection, or enhancing epithelial cell integrity, epithelial cell proliferation, epithelial cell differentiation, epithelial cell migration or epithelial wound healing in the intestine, of a subject in need thereof.

In one aspect, the present disclosure refers to the use of the IL-22 Fc fusion protein disclosed herein or the pharmaceutical composition disclosed herein in the manufacture of a medicament for treating acute kidney injury or acute pancreatitis in a subject in need thereof.

In one aspect, the present disclosure refers to the use of the IL-22 Fc fusion protein disclosed herein or the pharmaceutical composition disclosed herein in the manufacture of a medicament for accelerating or improving wound healing in a subject in need thereof.

In another example, a method for preventing or treating a cardiovascular condition, which condition includes a pathology of atherosclerotic plaque formation, is provided. The method includes administering to a subject in need thereof a therapeutically effective amount of an IL-22 polypeptide or an IL-22 Fc fusion protein. The cardiovascular condition includes, for

example, coronary artery disease, coronary microvascular disease, stroke, carotid artery disease, peripheral arterial disease, and chronic kidney disease. The method can include further slowing down the progression of atherosclerotic plaque formation. The method can further include administering one or more additional therapeutic agent to the subject for the prevention or treatment of the cardiovascular condition.

In one aspect, the present disclosure refers to the use of the IL-22 Fc fusion protein disclosed herein or the pharmaceutical composition disclosed herein in the manufacture of a medicament for preventing or treating a cardiovascular condition in a subject in need thereof, which condition includes a pathology of atherosclerotic plaque formation.

In another example, a method for treating metabolic syndrome is provided. The method includes administering to a subject in need thereof a therapeutically effective amount of an IL-22 polypeptide or an IL-22 Fc fusion protein. The method can further include reducing one or more risk factors associated with metabolic syndrome, including one or more of abdominal obesity, hyperglycemia, dyslipidemia, and hypertension. The method can further include reducing the level of bacterial lipopolysaccharide (LPS) in the subject. The method can further include administering one or more additional agent to the subject for the prevention or treatment of metabolic syndrome.

In one aspect, the present disclosure refers to the use of the IL-22 Fc fusion protein disclosed herein or the pharmaceutical composition disclosed herein in the manufacture of a medicament for treating metabolic syndrome in a subject in need thereof.

In another example, a method for delaying or slowing down the progression of atherosclerosis is provided. The method includes administering to a subject in need thereof a therapeutically effective amount of an IL-22 polypeptide or an IL-22 Fc fusion protein. The method can further include administering one or more additional agent to the subject for delaying or slowing down the progression of atherosclerosis.

In another example, a method of preventing indicia of atherosclerosis is provided. The method includes administering a therapeutically effective amount of an IL-22 polypeptide or an IL-22 Fc fusion protein to a subject at risk of atherosclerosis, wherein the IL-22 polypeptide of IL-22 Fc fusion protein is effective against the development of indicia of atherosclerosis. In certain examples, the subject has been identified to be at risk to develop a cardiovascular condition. In certain examples, the subject is genetically at risk of developing a cardiovascular condition. In one or more examples, the indicia of atherosclerosis include plaque accumulation. In some examples, the indicia of atherosclerosis include vascular inflammation. The method can further include administering one or more additional agent to the subject for preventing indicia of atherosclerosis.

In yet another example, a method of treating one or more of acute endotoxemia and sepsis is provided. The method includes administering to a subject in need thereof a therapeutically effective amount of an IL-22 polypeptide or an IL-22 Fc fusion protein. The method can further include administering one or more additional agent to the subject for treating one or more of acute endotoxemia and sepsis.

In one aspect, the present disclosure refers to the use of the IL-22 Fc fusion protein disclosed herein or the pharmaceutical composition disclosed herein in the manufacture of a medicament for treating acute endotoxemia, sepsis, or both, in a subject in need thereof.

In one other example, a method is provided for accelerating or improving wound healing, or both, in a subject. The method includes administering to a subject in need thereof a therapeutically effective amount of an IL-22 polypeptide, an IL-22 Fc fusion protein or an IL-22 agonist. In certain examples, the wound is a chronic wound. In certain other examples, the wound is an infected wound. In certain examples, the subject is diabetic, including a subject with type II diabetes. In one or more examples, the wound is a diabetic foot ulcer. In certain examples, the therapeutically effective amount of an IL-22 polypeptide, IL-22 Fc fusion protein or IL-22 agonist is administered until there is complete wound closure. In some examples, the administration is systemic; and in other examples, the administration is topical. In certain examples, the IL-22 polypeptide, IL-22 Fc fusion protein or IL-22 agonist is in a formulation for topical administration. In certain examples, the topical formulation comprises an IL-22 polypeptide without an Fc fusion. In certain examples, the IL22 agonist is selected from the group consisting of an IL-22 polypeptide, an IL-22 Fc fusion protein, an IL-22 agonist, an IL-19 polypeptide, an IL-19 Fc fusion protein, an IL-19 agonist, an IL-20 polypeptide, an IL-20 Fc fusion protein, an IL-20 agonist, an IL-24 polypeptide, an IL-24 Fc fusion protein, an IL-24 agonist, an IL-26 polypeptide, an IL-26 Fc fusion protein, an IL-26 agonist, and an IL-22R1 agonist. In certain other examples, the IL-22 agonist is selected from the group consisting of an IL-22 polypeptide, an IL-22 Fc fusion protein, an IL-22 agonist, an IL-20 polypeptide, an IL-20 Fc fusion protein, an IL-20 agonist, an IL-24 polypeptide, an IL-24 Fc fusion protein, an IL-24 agonist and an IL-22R1 agonist. In certain examples, the IL-22R1 agonist is an anti-IL22R1 agonistic antibody.

In a further example, the disclosure provides methods of treating a metabolic syndrome comprising the step of administering to a subject in need thereof a therapeutically effective amount of one or more IL-22 agonists. In certain examples, the IL22 agonist is selected from the group consisting of an IL-22 polypeptide, an IL-22 Fc fusion protein, an IL-22 agonist, an IL-19 polypeptide, an IL-19 Fc fusion protein, an IL-19 agonist, an IL-20 polypeptide, an IL-20 Fc fusion protein, an IL-20 agonist, an IL-24 polypeptide, an IL-24 Fc fusion protein, an IL-24 agonist, an IL-26 polypeptide, an IL-26 Fc fusion protein, an IL-26 agonist, and an IL-22R1 agonist. In certain other examples, the IL-22 agonist is selected from the group consisting of an IL-22 polypeptide, an IL-22 Fc fusion protein, an IL-22 agonist, an IL-20 polypeptide, an IL-20 Fc fusion protein, an IL-20 agonist, an IL-24 polypeptide, an IL-24 Fc fusion protein, an IL-24 agonist and an IL-22R1 agonist. In certain examples, the IL-22R1 agonist is an anti-IL22R1 agonistic antibody. In certain other examples, the metabolic syndrome is diabetes. In certain particular examples, the metabolic syndrome is type II diabetes.

According to another example, the subject is administered an IL-22 Fc fusion protein of the disclosure. In certain examples, the subject is a human. In certain examples, the IL-22 polypeptide or IL22 Fc fusion protein is administered intravenously, subcutaneously, intraperitoneally, systemically or topically.

In certain examples of these examples, the Fc region of the IL-22 Fc fusion protein is not glycosylated. In certain examples, the N297 residue and/or the T299 residue of the Fc region is changed. In certain examples, the N297 residue of the Fc region is changed. In certain other examples, the N297 residue is changed to Gly or Ala, preferably Gly. In certain other examples, the T299 residue is changed, preferably to Val, Gly or Ala. In certain particular examples, the IL-22 Fc fusion protein comprises the amino acid sequence of SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or EQ ID NO:14, preferably SEQ ID NO:8. In certain examples, the IL-22 Fc fusion protein is produced in E. coli. In certain examples, the subject is a human. In certain other examples, the pharmaceutical composition is administered intravenously, subcutaneously or topically.

In certain other examples, the pharmaceutical composition comprising IL-22 Fc fusion proteins has an afucosylation level in the CH2 domain of no more than 5%, preferably no more than 2%, more preferably less than 1%. In certain particular examples, the IL-22 Fc fusion protein comprises the amino acid sequence of SEQ ID NO:24 or SEQ ID NO:26, preferably SEQ ID NO:24. In certain other examples, the IL-22 Fc fusion protein is produced in CHO cells. In certain particular examples, the subject is a human. In certain other examples, the pharmaceutical composition is administered intravenously, subcutaneously or topically.

In yet other examples of the above examples, the N-glycan attached to the Fc region of the IL-22 Fc fusion protein is enzymatically removed by a glycolytic enzyme. In certain examples, the glycolytic enzyme is peptide-N-glycosidase (PNGase). In certain particular examples, the subject is a human.

In yet a further example, the disclosure also provides uses of an IL-22 Fc fusion protein described herein in the preparation of a medicament for the treatment of IBD, including UC and CD, in a subject in need thereof. In a related example, the disclosure provides uses of an IL-22 Fc fusion protein described herein in the preparation of a medicament for inhibiting microbial infection in the intestine, or preserving goblet cells in the intestine during a microbial infection in a subject in need thereof. In yet another example, the disclosure provides uses of an IL-22 Fc fusion protein described herein in the preparation of a medicament for enhancing epithelial cell integrity, epithelial cell proliferation, epithelial cell differentiation, epithelial cell migration or

epithelial wound healing in the intestine, in a subject in need thereof. In other related examples, the disclosure provides uses of an IL-22 polypeptide or IL-22 Fc fusion protein in the preparation of a medicament for treating a cardiovascular condition, metabolic syndrome, atherosclerosis, acute kidney injury, acute pancreatitis, accelerating, promoting or improving wound healing, including without limitation, healing of a chronic wound, diabetic wound, infected wound, pressure ulcer or diabetic foot ulcer, in a subject in need thereof.

Each and every example can be combined unless the context clearly suggests otherwise. Each and every example can be applied to the disclosure unless the context clearly suggests otherwise.

Specific examples of the present disclosure will become evident from the following more detailed description of certain preferred examples and the claims.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows amino acid sequence alignment of mature IL-22 from different mammalian species: human (GenBank Accession No.Q9GZX6, SEQ ID NO:4, chimpanzee (GenBank Accession No.XP_003313906, SEQ ID NO:48), orangutan (GenBank Accession No. XP_002823544, SEQ ID NO:49), mouse (GenBank Accession No. Q9JJY9, SEQ ID NO:50) and dog (GenBank Accession No. XP_538274, SEQ ID NO:51).

Figure 2 shows mass spectrometry results of the glycosylation status of the Fc region of a typical human monoclonal IgG1 Fc (Panel A), IL-22 IgG1 Fc fusion containing the linker sequence EPKSCDKTHT (SEQ ID NO:33, Panel B), EPKSSDKTHT (SEQ ID NO:40, Panel C), and GGGDKTHT (SEQ ID NO:41, Panel D), and IL-22 IgG4 Fc fusion containing the linker sequence RVESKYGPP without or with the N297G mutation (SEQ ID NO:44, Panels E and F, respectively) and IL-22 IgG1 Fc fusion containing the linker sequence EPKSSDKTHT (SEQ ID NO:40) with the N297G mutation (Panel G).

Figure 3 shows sequence alignment of human IL-22 IgG4 Fc fusion (N297G, full length Fc sequence with the C-terminal Lys, SEQ ID NO:16, without Lys SEQ ID NO:8), IL-22 IgG1 Fc fusion (N297G, full length Fc sequence with the C-terminal Lys, SEQ ID NO:20, without Lys SEQ ID NO:12) and IL-22 (SEQ ID NO:4). The IL-22 sequence shown is the matured form without the leader sequence. The hinge sequence CPPCP (SEQ ID NO:31) is shown in the box, followed by the CH2 and CH3 domains. The N297G substitution and the optional C-terminus Lys residue are marked.

Figure 4 presents a graph showing the results of STAT3 luciferase assay. Luciferase activity stimulated by IL-22 IgG4 Fc fusion or IL-22 IgG1 Fc fusion was measured in 293 cells

WHAT IS CLAIMED IS:

1. An IL-22 Fc fusion protein comprising an IL-22 polypeptide linked to an IgG Fc region by a linker, wherein the Fc region comprises an IgG1 Fc region or an IgG4 Fc region, and wherein the Fc region is not glycosylated.

2. The IL-22 Fc fusion protein of claim 1, wherein the Fc region comprises a hinge region comprising the amino acid sequence of CPPCP (SEQ ID NO:31).

3. The IL-22 Fc fusion protein of claim 1 or 2, wherein the Fc region comprises an altered glycosylation consensus site.

4. The IL-22 Fc fusion protein of claim 3, wherein the Fc region comprises an insertion, a deletion, or a substitution mutation that results in an aglycosylated Fc region.

5. The IL-22 Fc fusion protein of any one of claims 1-4, wherein in the Fc region the amino acid residue at position 297 (numbering according to the EU index) of the Fc region is changed and/or the amino acid residue at position 299 (numbering according to the EU index) of the Fc region is changed.

6. The IL-22 Fc fusion protein of claim 5, wherein the amino acid residue at position 297 (numbering according to the EU index) of the Fc region is Gly, Ala, Gln, Asp, or Glu.

7. The IL-22 Fc fusion protein of claim 6, wherein the amino acid residue at position 297 (numbering according to the EU index) of the Fc region is Gly or Ala.

8. The IL-22 Fc fusion protein of claim 6 or 7, wherein the amino acid residue at position 297 (numbering according to the EU index) of the Fc region is Gly.

9. The IL-22 Fc fusion protein of any one of claims 5-8, wherein the amino acid residue at position 299 (numbering according to the EU index) is Ala, Gly or Val.

10. The IL-22 Fc fusion protein of any one of claims 1-9, wherein the linker is 8-20 amino acids long.

11. The IL-22 Fc fusion protein of claim 10, wherein the linker is 8-16 amino acids long.

12. The IL-22 Fc fusion protein of claim 10, wherein the linker is 10-16 amino acids long.

13. The IL-22 Fc fusion protein of any one of claims 1-12, wherein the Fc region is an IgG1 Fc region.

14. The IL-22 Fc fusion protein of claim 13, wherein the linker comprises the amino acid sequence DKTHT (SEQ ID NO:32).

15. The IL-22 Fc fusion protein of claim 14, wherein the linker is at least 11 amino acids long and comprises the amino acid sequence EPKSCDKTHT (SEQ ID NO:33).

16. The IL-22 Fc fusion protein of claim 15, wherein the linker comprises the amino acid sequence VEPKSCDKTHT (SEQ ID NO:34), KVEPKSCDKTHT (SEQ ID NO:35), KKVEPKSCDKTHT (SEQ ID NO:36), DKKVEPKSCDKTHT (SEQ ID NO:37), VDKKVEPKSCDKTHT (SEQ ID NO:38), or KVDKKVEPKSCDKTHT (SEQ ID NO:39).

17. The IL-22 Fc fusion protein of any one of claims 1-14, wherein the linker comprises the amino acid sequence EPKSSDKTHT (SEQ ID NO:40).

18. The IL-22 Fc fusion protein of claim 17, wherein the linker comprises the amino acid sequence VEPKSSDKTHT (SEQ ID NO:67), KVEPKSSDKTHT (SEQ ID NO:68), KKVEPKSSDKTHT (SEQ ID NO:66), DKKVEPKSSDKTHT (SEQ ID NO:64), VDKKVEPKSSDKTHT (SEQ ID NO:69), or KVDKKVEPKSSDKTHT (SEQ ID NO:65).

19. The IL-22 Fc fusion protein of claim 1 or 14, wherein the linker does not comprise the amino acid sequence GGS (SEQ ID NO:45).

20. The IL-22 Fc fusion protein of any one of claims 1-14, 17, and 19, comprising an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:12.

21. The IL-22 Fc fusion protein of claim 20, comprising an amino acid sequence having at least 96% sequence identity to the amino acid sequence of SEQ ID NO:12.

22. The IL-22 Fc fusion protein of claim 21, comprising an amino acid sequence having at least 97% sequence identity to the amino acid sequence of SEQ ID NO:12.

23. The IL-22 Fc fusion protein of claim 22, comprising an amino acid sequence having at least 98% sequence identity to the amino acid sequence of SEQ ID NO:12.

24. The IL-22 Fc fusion protein of claim 23, comprising an amino acid sequence having at least 99% sequence identity to the amino acid sequence of SEQ ID NO:12.

25. An IL-22 Fc fusion protein comprising the amino acid sequence of SEQ ID NO:12.

26. An IL-22 Fc fusion protein comprising the amino acid sequence of SEQ ID NO:14.

27. An IL-22 Fc fusion protein comprising the amino acid sequence of SEQ ID NO:20.

28. An IL-22 Fc fusion protein consisting of the amino acid sequence of SEQ ID NO:12.

29. An IL-22 Fc fusion protein consisting of the amino acid sequence of SEQ ID NO:14.

30. An IL-22 Fc fusion protein consisting of the amino acid sequence of SEQ ID NO:20.

31. The IL-22 Fc fusion protein of any one of claims 1-12, wherein the Fc region comprises an IgG4 Fc region.

32. The IL-22 Fc fusion protein of claim 31, wherein the linker comprises the amino acid sequence SKYGPP (SEQ ID NO:43).

33. The IL-22 Fc fusion protein of claim 32, wherein the linker comprises the amino acid sequence RVESKYGPP (SEQ ID NO:44).

34. The IL-22 Fc fusion protein of any one of claims 1-33 produced by a method comprising the step of culturing a host cell capable of expressing the IL-22 Fc fusion protein under conditions suitable for expression of the IL-22 Fc fusion protein.

35. The IL-22 Fc fusion protein of claim 34, wherein the method further comprises the step of obtaining the IL-22 Fc fusion protein from the cell culture or culture medium.

36. The IL-22 Fc fusion protein of claim 34 or 35, wherein the host cell is a CHO cell.

37. The IL-22 Fc fusion protein of any one of claims 1-36, wherein the IL-22 fusion protein is a dimeric IL-22 Fc fusion protein.

38. The IL-22 Fc fusion protein of any one of claims 1-36, wherein the IL-22 fusion protein is a monomeric IL-22 Fc fusion protein.

39. The IL-22 Fc fusion protein of any one of claims 1-38, wherein the IL-22 polypeptide comprises the amino acid sequence of SEQ ID NO:4.

40. The IL-22 Fc fusion protein of any one of claims 1-39, wherein the IL-22 polypeptide is a human IL-22 polypeptide.

41. The IL-22 Fc fusion protein of any one of claims 1-40, wherein the IL-22 Fc fusion protein binds to IL-22 receptor.

42. The IL-22 Fc fusion protein of claim 41, wherein the IL-22 receptor is a human IL-22 receptor.

43. An isolated nucleic acid encoding the IL-22 Fc fusion protein of any one of claims 1-42.

44. The isolated nucleic acid of claim 43, comprising the polynucleotide sequence of SEQ ID NO:11

45. The isolated nucleic acid of claim 43, comprising the polynucleotide sequence of SEQ ID NO:13.

46. The isolated nucleic acid of claim 43, comprising the polynucleotide sequence of SEQ ID NO:19.

47. A vector comprising the nucleic acid of any one of claims 43-46.

48. A host cell comprising the vector of claim 47.

49. The host cell of claim 48, wherein the host cell is a prokaryotic cell or eukaryotic cell.

50. The host cell of claim 49, wherein the host cell is an *E. coli* cell or a CHO cell.

51. The host cell of claim 50, wherein the host cell is a CHO cell.

52. A method of making an IL-22 Fc fusion protein comprising the step of culturing the host cell of any one of claims 48-51 under conditions suitable for expression of the IL-22 Fc fusion protein.

53. The method of claim 52, further comprising the step of obtaining the IL-22 Fc fusion protein from the cell culture or culture medium.

54. The method of claim 53, wherein the host cell is a prokaryotic cell or a eukaryotic cell.

55. The method of any one of claims 52-54, wherein the host cell is an *E. coli* cell.

56. The method of any one of claims 52-54, wherein the host cell is a CHO cell.

57. A pharmaceutical composition comprising the IL-22 Fc fusion protein according to any one of claims 1-42 and at least one pharmaceutically acceptable carrier.

58. The pharmaceutical composition of claim 57, further comprising an additional therapeutic agent.

59. A pharmaceutical composition comprising an IL-22 polypeptide or an IL-22 Fc fusion protein of any one of claims 1-42 and at least one pharmaceutically acceptable carrier, wherein the pharmaceutically acceptable carrier is a gelling agent.

60. The pharmaceutical composition of claim 59, wherein the gelling agent is a polysaccharide.

61. The pharmaceutical composition of claim 59 or 60, wherein the gelling agent is a cellulosic agent.

62. The pharmaceutical composition of any one of claims 59-61, wherein the gelling agent is methylcellulose, hydroxyethyl cellulose, carboxymethyl cellulose, hydroxypropyl cellulose, POE-POP block polymers, alginate, hyaluronic acid, polyacrylic acid, hydroxyethyl methylcellulose or hydroxypropyl methylcellulose.

63. The pharmaceutical composition of claim 62, wherein the gelling agent is hydroxypropyl methylcellulose.

64. The pharmaceutical composition of any one of claims 59-63, wherein the pharmaceutical composition is for topical administration

65. Use of the IL-22 Fc fusion protein of any one of claims 1-43 or the pharmaceutical composition of claim 57 or 58 in the manufacture of a medicament for treating inflammatory bowel disease (IBD) in a subject in need thereof.

66. The use of claim 65, wherein the IBD is ulcerative colitis.

67. The use of claim 65, wherein the IBD is Crohn's disease.

68. Use of the IL-22 Fc fusion protein of any one of claims 1-43 or the pharmaceutical composition of claim 57 or 58 in the manufacture of a medicament for inhibiting microbial infection in the intestine, preserving goblet cells in the intestine during a microbial infection, or enhancing epithelial cell integrity, epithelial cell proliferation, epithelial cell differentiation, epithelial cell migration or epithelial wound healing in the intestine, of a subject in need thereof.

69. The use of claim 68, wherein the epithelial cell is intestinal epithelial cell.

70. Use of the IL-22 Fc fusion protein of any one of claims 1-43 or the pharmaceutical composition of claim 57 or 58 in the manufacture of a medicament for treating acute kidney injury or acute pancreatitis in a subject in need thereof.

71. Use of the IL-22 Fc fusion protein of any one of claims 1-43 or the pharmaceutical composition of any one of claims 57-64 in the manufacture of a medicament for accelerating or improving wound healing in a subject in need thereof.

72. The use of claim 71, wherein the wound is a chronic wound or an infected wound.

73. The use of claim 71 or 72, wherein the subject is diabetic.

74. The use of claim 73, wherein the diabetic subject has type II diabetes.

75. The use of any one of claims 71-74, wherein the wound is a diabetic foot ulcer.

76. The use of any one of claims 71-75, wherein the pharmaceutical composition is to be administered until there is complete wound closure.

77. Use of the IL-22 Fc fusion protein of any one of claims 1-43 or the pharmaceutical composition of claim 57 or 58 in the manufacture of a medicament for preventing or treating a cardiovascular condition in a subject in need thereof, which condition includes a pathology of atherosclerotic plaque formation.

78. The use of claim 77, wherein the cardiovascular condition is selected from the group consisting of coronary artery disease, coronary microvascular disease, stroke, carotid artery disease, peripheral arterial disease, and chronic kidney disease.

79. The use of claim 77 or 78, further comprising slowing down the progression of atherosclerotic plaque formation or preventing indicia of atherosclerosis.

80. The use of claim 79, wherein the indicia of atherosclerosis includes plaque accumulation or vascular inflammation.

81. The use of any one of claims 77-80, wherein the subject has been identified to be at risk to develop a cardiovascular condition.

82. Use of the IL-22 Fc fusion protein of any one of claims 1-43 or the pharmaceutical composition of claim 57 or 58 in the manufacture of a medicament for treating metabolic syndrome in a subject in need thereof.

83. The use of claim 82, further comprising reducing one or more risk factors associated with metabolic syndrome, including one or more of abdominal obesity, hyperglycemia, dyslipidemia, and hypertension.

84. The use of claim 82 or 83, further comprising reducing the level of bacterial lipopolysaccharide (LPS) in the subject.

85. The use of any one of claims 82-84, wherein the subject is in need of a change in HDL/LDL lipid profile.

86. Use of the IL-22 Fc fusion protein of any one of claims 1-43 or the pharmaceutical composition of claim 57 or 58 in the manufacture of a medicament for treating acute endotoxemia, sepsis, or both, in a subject in need thereof.

87. The use of claim 86, wherein the subject is in need of a change in HDL/LDL lipid profile.

88. The use of any one of claims 65-87, wherein the subject is to be administered at least one additional therapeutic agent.

89. The use of any one of claims 65-88, wherein the IL-22 Fc fusion protein or the pharmaceutical composition is to be administered intravenously, subcutaneously, or intraperitoneally.

90. The use of any one of claims 65-89, wherein the IL-22 Fc fusion protein or the pharmaceutical composition is to be administered intravenously.

91. The use of any one of claims 65-89, wherein the IL-22 Fc fusion protein or the pharmaceutical composition is to be administered subcutaneously.

92. The use of any one of claims 65-91, wherein the subject is a patient.

93. The use of any one of claims 65-92, wherein the subject is a human.