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(71) Applicant: **AVALON BIOLOGICS LIMITED** [CN/CN]; c/o IC Development Centre, Unit 608-613 - No.6 Science Park West Avenue, HKSTP, Sha Tin, New Territories, Hongkong (CN).

(72) Inventors: **YU, Kuo-ming**; 2/F No.63, Mei-Lune St, Shil-in District, Taipei (TW). **CHOO, Qui-lim**; 5700 Fern Street, El Cerrito, CA 94530 (US). **FOK, Manson**; Pedder Clinic, 4th Floor, 9 Queen's Road Central, Hong Kong (CN). **LAU, Johnson, Yiu-nam**; 9 Seabluff, Newport Beach, CA 92660 (US).

(74) Agents: **FESSENMAIER, Martin** et al.; Fish&Tsang, LLP, 2603 Main Street, Ste 1000, Irvine, CA 92614 (US).

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(54) Title: COMPOSITIONS AND METHODS FOR PEGYLATED IL-11

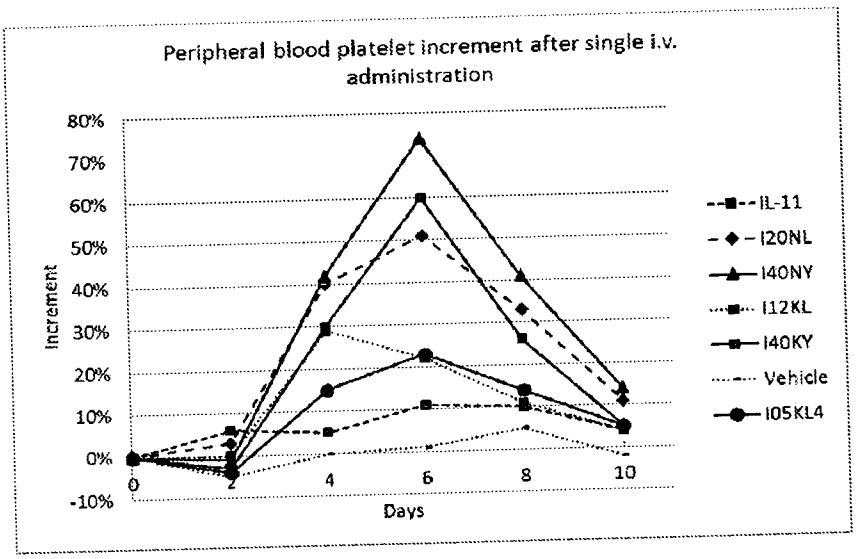


Figure 4

(57) Abstract: Compositions and methods are presented in which recombinant IL-11 is PEGylated to achieve improved half-life in serum while having desirable therapeutic activity and presenting less side-effects. Most preferably, the IL-11 is an N-terminally truncated human or humanized IL-11 and has a 20Kd or 40Kd branched PEG moiety, Y- or comb- shaped in particular, coupled to the N-terminal amino group. Such compounds are characterized by substantially increased stability in serum and sustained biological activity while exhibiting significantly reduced plasma expansion.

WO 2016/140983 A1

COMPOSITIONS AND METHODS FOR PEGYLATED IL-11

[0001] This application claims priority to our copending US provisional application with the serial number 62/127748, which was filed 03-Mar-15, and which is incorporated by reference herein.

Field of the Invention

[0002] The field of the invention is pharmaceutical compositions and methods, especially as they relate to PEGylated Interleukin 11 (IL-11).

Background of the Invention

[0003] The background description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed invention, or that any publication specifically or implicitly referenced is prior art. All publications herein are incorporated by reference to the same extent as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Where a definition or use of a term in an incorporated reference is inconsistent or contrary to the definition of that term provided herein, the definition of that term provided herein applies and the definition of that term in the reference does not apply.

[0004] Chemotherapy-induced thrombocytopenia remains an unmet medical need because the current treating regimen employs transfusion of platelets that may be in short supply and carry a risk of viral contamination. On the other hand, recombinant human IL-11 can be given to a patient to stimulate platelet production. However IL-11 administration requires daily dosing, leading to marginal clinical efficacy and plasma expansion.

[0005] IL-11 is a cytokine and acts as a major signaling agent in hematopoiesis, and especially in the stimulation of megakaryocyte maturation. Action of IL-11 is typically mediated by the IL-11 receptor and glycoprotein gp130 with subsequent phosphorylation/activation of gp130. Clinical uses for IL-11 include treatment of side effects associated with chemotherapy, which is thought to enhance megakaryocytopoiesis and increase platelet counts. Recombinant human IL-11 is commercially available as NEUMEGA® (Oprelvekin, Wyeth-Ayerst) and is approved for the prevention of severe thrombocytopenia and the reduction of the need for platelet transfusions following myelosuppressive chemotherapy in adult patients with non-myeloid

malignancies who are at high risk of severe thrombocytopenia. NEUMEGA® is typically supplied in a single use vial containing 5 mg IL-11 as a lyophilized powder for reconstitution with 1 mL sterile water for injection (administered at a dose of 25-50 μg/kg/day). The most frequent adverse event associated with NEUMEGA™ is plasma expansion leading to life-threatening atrial arrhythmias, syncope, dyspnea, congestive heart failure, and pulmonary edema.

[0006] IL-11 is cleared from the circulatory system relatively quickly and as such requires multiple injections. For example, Neumega™ subcutaneously administrated in healthy men has a terminal half-life about 6.9 hours (Product Insert of Neumega™). The poor pharmacokinetics such as rapid renal excretion and proteolytic digestion, as well as its associated adverse effect often reduce clinical prevalence. Moreover, daily injection also means hospitalization to manage adverse event that not only adds on medical expense but also damages quality of life to patients. As a result, platelet transfusion remains the gold-standard for treating chemotherapy-induced thrombocytopenia (CIT).

[0007] Several attempts have been undertaken in the art to increase serum stability while maintaining beneficial therapeutic potential of such compositions. For example, US 2010/0098658 reports an IL-11 analog (mIL-11) in association with a polymer (PEG) that exhibited enhanced resistance to acidolysis and increased serum half-life. In another attempt to stabilize IL-11, as described in US8133480, cysteine variants of IL-11 were prepared and selected muteins were further modified with PEG to increase serum stability. While these modifications have improved serum stability or half-life of IL-11 to at least some degree, one or more disadvantages nevertheless remain, including marginal efficacy in myelosuppressive animals, complexity in production, repeated dosing, and formulation into injectable solution.

[0008] Because of lacking cysteine residues in IL-11, the '480 patent describes insertion of a cysteine residue in the C-terminal amino acid sequence, conferring a functional group to allow conjugation of a thiol-reactive polyethylene glycol chain. Although the biological activity was conserved, the introduction of cysteine may yield intermolecular dimers and the production yield of insect cells may be lower than bacterial production. Additionally the serum half-life of so modified IL-11 when administrated intravenously in male Sprague-Dawley rats was about 5.6 hr for 40 KD PEGylation, which is less than desirable. Moreover, in animal studies using cyclophosphamide-treated rats, the efficacy was marginal with the-other-day dosing scheme. Another employed PEG conjugation onto N-terminally truncated sequence of IL-11 with 20

KD PEG via amine or amide bonding was described in US 2010/0098658. Although the N-terminal truncation did not reduce its biological activity, the serum half-life administered subcutaneously in male Sprague-Dawley rats was about 8.5 hr, again falling short of desirable stability. Additionally, the efficacy in an animal disease model was unknown.

[0009] Linear or branched PEG of 20 KD conjugating onto amine groups of IL-11 was reported (Takagi et al. 2007, "Enhanced pharmacological activity of recombinant human interleukin-11 (rhIL11) by chemical modification with polyethylene glycol." *J Control Release*, 119(3):271-278), such unspecific conjugation often resulted in multiple PEGylation via reaction with lysine, histidine, and tyrosine residues as well as N-terminal amines.

[0010] Other reports have demonstrated certain carbohydrate modifications on the "non-core" regions of IL-11 such as N-terminus and loops enhanced cell-stimulatory activities, suggesting these regions are perhaps designed to limit biological activity of IL-11 (Yanaka et al. 2011, "Non-core region modulates interleukin-11 signaling activity: generation of agonist and antagonist variants." *J. Biol. Chem.*, 286:8085-8093). However, no desirable modification was reported with stabilities and activities above unmodified IL-11.

[0011] Thus, even though several methods of stabilizing IL-11 are known in the art, all or almost all have one or more drawbacks, such as limited efficacy and requirement for repeated dosing. More importantly, even in modified form, adverse effects of IL-11 (e.g., plasma expansion) were not reduced. Therefore, there remains a need for improved compositions and methods to stabilize IL-11 while simultaneously alleviate adverse effects.

Summary of the Invention

[0012] The inventive subject matter is directed to compounds, compositions, and methods for improving stability and half-life time of IL-11 in serum while maintaining biological activity and mitigating side-effects. In especially preferred aspects, the inventors have discovered that the amino acid position, manner of attachment, and type of PEG is critical to producing stable and biologically active PEGylated IL-11, and particularly preferred PEGylated IL-11 will have the same sequence as native human IL-11 but lack the N-terminal first amino acid, proline. Moreover, such IL-11 will preferably be covalently modified at the N-terminus with a possible secondary site at certain lysine residues within the polypeptide chain. Most typically, the average molar ratio of IL-11 to PEG compound attached to the IL-11 is 1:1.

[0013] In one aspect of the inventive subject matter, the inventors contemplate a modified interleukin 11 (IL-11) compound that includes an IL-11 polypeptide chain that is covalently coupled to a PEG moiety, wherein the PEG moiety has an average molecular weight of between 10-50Kd and has distinct first and second PEG portions, wherein the PEG moiety is covalently bound to an N-terminal amino acid, and wherein the IL-11 polypeptide chain is a human or humanized polypeptide chain.

[0014] Most typically, the IL-11 polypeptide chain is a human IL-11 polypeptide chain, and/or maybe shortened by deletion of an N-terminal proline. For example, especially suitable IL-11 polypeptide chain may have a sequence according to SEQ ID NO:1. With respect to the PEG moiety it is generally preferred that the moiety has an average molecular weight of 20Kd or 40Kd, and/or that the PEG moiety has a Y shape. While not limiting to the inventive subject matter, it is preferred that the molar ratio of polypeptide chain to PEG moiety is about 1:1 (*e.g.*, 0.9:1 to 1:0.9, or 0.8:1 to 1:0.8). In addition, it is contemplated that a second PEG moiety may be covalently coupled to the modified IL-11 via an amino group of the IL-11 polypeptide chain. Furthermore, it is generally preferred that the PEG moiety is covalently bound to the N-terminal amino acid via an amine bond (however, amide bonds are also specifically contemplated).

[0015] Viewed from another perspective, the inventors also contemplate a pharmaceutical composition that include a therapeutically effective amount of an IL-11 compound according to the inventive subject matter (*e.g.*, as described above), in combination with a pharmaceutically acceptable carrier. Where desirable, the composition may be formulated for injection, and may include the IL-11 compound is present in an amount to provide a dosage unit of between 10-100 µg/kg for a pediatric or adult patient. Additionally, it is contemplated that the composition may be lyophilized, or in a liquid form for injection or infusion. As best suitable, the pharmaceutical composition may further include a second pharmaceutically active compound, separately, or in admixture with the IL-11 compound. Thus, kits comprising contemplated pharmaceutical compositions together with other components (*e.g.*, second pharmaceutically active compound such as a steroid, an agent that stimulates platelet production in bone marrow, an antibody, an analgesic, or anti-inflammatory agent, or a solvent for reconstitution) are also expressly contemplated herein

[0016] Consequently, the inventors also contemplate use of an IL-11 compound according to the inventive subject matter in the manufacture of a pharmaceutical composition. While not

limiting to the inventive subject matter, especially contemplated treatments include (a) nuclear accident/radiation induced bone and gastrointestinal damage; (b) chemotherapy induced bone and gastrointestinal damage; (c) burn induced thrombocytopenia and gastrointestinal damage; (d) chemotherapy induced thrombocytopenia; (e) trauma-, cancer-, or infection-induced gastrointestinal damage or inflammatory bowel disease, (f) free radical-induced lung damage, and (g) cardiovascular diseases. As noted before, it is generally contemplated that the pharmaceutical composition is formulated for injection and/or that the pharmaceutical composition is lyophilized.

[0017] In a further aspect of the inventive subject matter, the inventors therefore also contemplate a method of increasing serum half-life of an interleukin 11 (IL-11) compound. Preferred methods will include a step of covalently coupling an IL-11 polypeptide chain to a PEG moiety, wherein the PEG moiety has an average molecular weight of between 10-50Kd and has distinct first and second PEG portions, wherein the PEG moiety is covalently bound to an N-terminal amino acid, and wherein the IL-11 polypeptide chain is a human or humanized polypeptide chain. Most typically, the IL-11 polypeptide chain is a human IL-11 polypeptide chain, and/or the IL-11 polypeptide chain is shortened by deletion of an N-terminal proline (*e.g.*, having a sequence according to SEQ ID NO:1).

[0018] In further contemplated methods, the PEG moiety has an average molecular weight of 20Kd or 40Kd, and/or may have a Y shape. Where desired, the molar ratio of the polypeptide chain to the PEG moiety is about 1:1, and it is further contemplated that the methods may further include a step of covalently coupling a second PEG moiety via an amino group in the IL-11 polypeptide chain. As before, it is contemplated that the PEG moiety is covalently bound to the N-terminal amino acid via an amine bond.

[0019] In further contemplated methods, the inventors contemplate a method of treating a condition that is responsive to administration of IL-11. Such methods will typically include a step of administering contemplated pharmaceutical compositions in a therapeutically effective amount to a patient in need thereof. For example, suitable condition may be selected from the group consisting of (a) nuclear accident/radiation induced bone and gastrointestinal damage; (b) chemotherapy induced bone and gastrointestinal damage; (c) burn induced thrombocytopenia and gastrointestinal damage; (d) chemotherapy induced thrombocytopenia; (e) trauma-, cancer-, or infection-induced gastrointestinal damage or inflammatory bowel disease, (f) free radical-induced lung damage, and (g) a cardiovascular disease. Exemplary

preferred pharmaceutical composition for these methods may comprise IL-11 I40NY or I20NY, and it is further contemplated that IL-11 is administered (*e.g.*, subcutaneously) in a dosage between 10-100 µg/kg.

[0020] Various objects, features, aspects and advantages of the inventive subject matter will become more apparent from the following detailed description of preferred embodiments, along with the accompanying drawing figures in which like numerals represent like components.

Brief Description of The Drawing

[0021] Figure 1 depicts the primary sequence of IL-11 without N-terminal proline.

[0022] Figure 2 are images of SDS-PAGE gels with molecular weight markers and various PEGylated forms of IL-11 as indicated.

[0023] Figure 3 is a graph depicting plasma concentrations of various IL-11 compositions after single *i.v.* administration.

[0024] Figure 4 is a graph depicting platelet increment after single *i.v.* administration of various IL-11 compositions.

[0025] Figure 5 depicts chromatograms of peptide maps for tryptic digestion of unconjugated IL-11, I40NY and I40KY in comparison.

[0026] Figure 6 is a graph depicting platelet increment after *s.c.* administration of various IL-11 compositions (Daily injection for IL-11 in consecutive 14 days; and weekly injection for PEGylated counterparts).

[0027] Figure 7 is a graph depicting hematocrit reduction after *s.c.* administration of various IL-11 compositions (Daily injection for IL-11 in consecutive 14 days; and weekly injection for PEGylated counterparts).

[0028] Figure 8 is a graph suggesting a correlation between maximal platelet induction and maximal reduction of hematocrit.

[0029] Figure 9 are graphs depicting cell proliferation activity of PEGylated compounds in 7TD1 assays, in comparison to unconjugated IL-11.

[0030] Figure 10 is an image of a non-reducing SDS-PAGE gel with silver stain illustrating the purity of I40NY at various loading quantities.

[0031] Figure 11 is a HPLC chromatogram depicting the product purity of mono-PEGylated component for I40NY.

[0032] Figure 12 is a pharmacokinetic profile depicting the kinetics of the plasma concentration of I40NY after single *s.c.* administration, in comparison to single *s.c.* administration of unconjugated IL-11.

[0033] Figure 13 is an overlay of circular dichroism spectra of IL-11 and I40NY.

[0034] Figure 14 is an ellipticity plot for IL-11 and I40NY as a function of temperature.

[0035] Figure 15 is a pharmacodynamics profile depicting the platelet production of contemplated compounds in an animal model of myelosuppressive rats.

[0036] Figure 16 is a graph depicting the hematocrit reduction of contemplated compounds in an animal model of myelosuppressive rats.

Detailed Description

[0037] The inventors have discovered that the type of PEG compound, the location of covalent attachment, and the primary sequence of IL-11 are determinants to the stability and activity of so modified IL-11. In particularly preferred and unexpected aspects, the inventors discovered that IL-11 has substantially improved stability when truncated at the N-terminus by one amino acid that is then PEGylated. Furthermore, the inventors also discovered that the particular type and molecular weight of the PEG moiety is an additional determinant of stability, activity, and toxicity as is further described in more detail below.

Contemplated Compounds

[0038] To investigate the influence of type, molecular weight, and attachment position of PEG to IL-11, the inventors prepared various PEGylated IL-11 molecules from recombinant human IL-11 having a primary sequence as shown in **Figure 1** (identical with the native human IL-11 sequence, but lacking the N-terminal proline). It is generally preferred that the IL-11 protein is a N-terminal truncated or modified human IL-11. For example, especially preferred truncated forms include IL-11 molecules that lack at least one or two or three (or more) N-terminal amino

acids. Alternatively, the IL-11 may also be modified to have a N-terminal amino acid that is different from the human unmodified counterpart. For example, a modified IL-11 may lack the first N-terminal amino acid and may have a second amino acid that is other than the second amino acid found in unmodified human IL-11 (*e.g.*, lacking P and having G replaced by V). Most typically, N-terminal amino acids will be stabilizing amino acids and therefore especially include M, G, A, S, T, V, or P, and in further contemplated aspects, destabilizing amino acids (*e.g.*, F, Q, N, R, etc.) may be replaced by stabilizing amino acids. Deletions of one or more amino acids from the N-terminal end will typically be limited to the first ten, or the first five, or the first three amino acids. On the other hand, in less preferred aspects, deletions of one, two, three, four, five, or more amino acids may also be implemented at the C-terminus of the IL-11 moiety. As a general guidance, deletions will generally be limited to those that do not or only moderately adversely affect biological activity and/or stability (*e.g.*, loss of activity and/or stability is less than 20%, and more typically less than 10%). Alternatively, or additionally, contemplated IL-11 molecules also include fusion proteins with IL-11 an exemplary fusion proteins include those described in US 2010/0143973, which is incorporated by reference herein. Most typically, the IL-11 is a recombinant protein and may be expressed in a suitable expression system, and most preferably in a prokaryotic system (*e.g., E. coli*) or yeast system (*e.g., Pichia pasteuris*). Of course, it should also be recognized that particularly preferred forms of IL-11 are mature forms (*i.e.*, without leader sequence)

[0039] Moreover, it should be appreciated that suitable IL-11 molecules need not be human IL-11 but may be of any other (typically mammalian) origin. Therefore, suitable IL-11 sources (recombinant or native) include primate, murine, porcine, equine, etc. These sequences may then be at least partially humanized to reduce immunogenicity and/or increase stability and/or activity in human. Similarly, synthetic consensus sequences are also contemplated herein.

[0040] PEGylation of contemplated IL-11 molecules may be performed in numerous manners and includes covalent as well as non-covalent methods. However, it is generally preferred that the PEGylation uses covalent binding to the IL-11. There are numerous manners known in the art to covalently attach a PEG group to a protein and suitable methods include those that react the N-terminal amino group or the C-terminal carboxylic acid group with a suitable reactive group on the PEG moiety (*e.g.*, aldehyde, maleimide, acid chloride, etc.), or sulfhydryl reactive groups (*e.g.*, maleimide, pyridyl disulfide, vinyl sulfone, etc.) that allow for disulfide bonding to cysteine groups, or amino reactive reagents that react with an ε-amino group of a lysine

amino acid (*e.g.*, NHS-esters, NHS-carbonates, triazine, groups, etc). Therefore, it is also contemplated that one or more amino acids may be added to the N- and/or C-terminus to introduce a reactive group suitable for attachment of a PEGylation group. For example, serine or threonine may be added to allow for enzymatic attachment using a N-actylgalactosamine or PEG sialic derivative, or a lysine for covalent attachment to the ε-amino group, or a phenyl alanine or threonine group for attachment to a hydroxyl group.

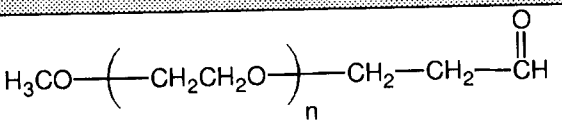
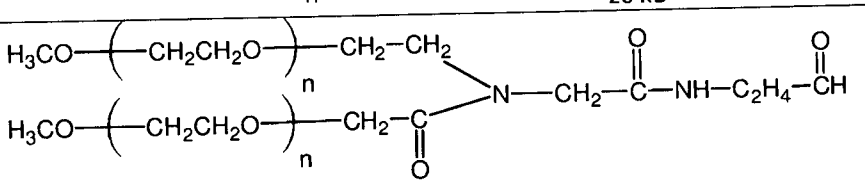
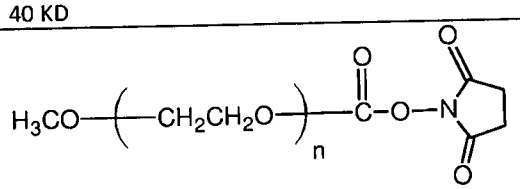
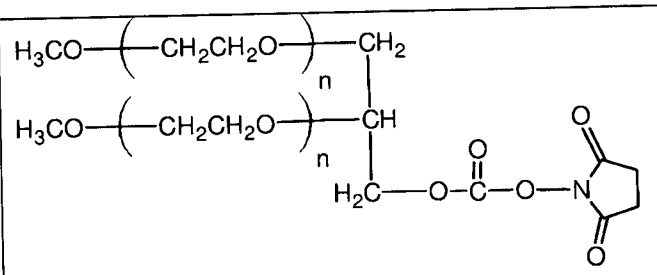
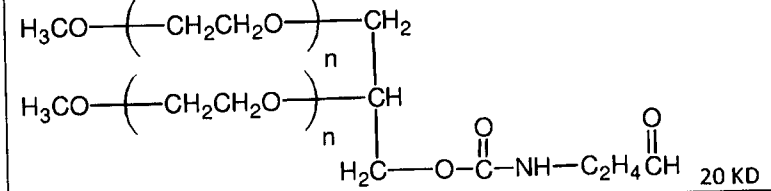
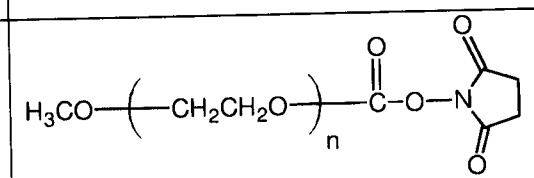
[0041] With respect to suitable PEG molecules for use herein, it is generally contemplated that various molecular weights for PEG are appropriate, and contemplated molecular weights are between 2 Kd and 200 Kd (average or nominal molecular weight). However, particularly preferred molecular weights (average or nominal molecular weight) include those between 10-50 Kd per linear chain in a PEGylation moiety. Moreover, it is generally preferred that the PEG moiety will have a single linear, or Y-shaped PEG moiety, and even more preferably that such PEG moiety will have a molecular weight of between 20-40 Kd. Alternatively, suitable PEG moieties may also include dendrimeric PEG constructs, as well as PEG moieties with more than two linear chains. Where the PEG moieties have more than one linear PEG chain, it is generally preferred that the chains have an average molecular weight that is substantially the same (average molecular weight difference less than 15%).

[0042] In further preferred aspects, the PEG moiety is covalently attached to the IL-11 via the N-terminal amino group of IL-11 and/or (optionally) to an ε-amino group of an internal lysine or the ring nitrogen of histidine. Due to the N-terminal covalent bond, it is preferred that the molar ratio of IL-11 to PEG moiety is about 1:1 (*e.g.*, 0.9:1 to 1:0.9, or 0.8:1 to 1:0.8, etc.). In addition, it should be appreciated that moderate levels of PEGylation may be present at internal amino acid residues (*e.g.* between 10%-20%, or between 1%-10% of all IL-11 may carry an extra PEGylated internal amino acid). For example, a second PEG moiety may be attached to an ε-amino group of an internal lysine or histidine. As is further shown in more detail below, a particularly preferred form of PEGylated IL-11 is I40NY, comprising human IL-11 (lacking N-terminal proline), to which is attached on the N-terminus a Y-shaped PEG moiety with an average molecular weight of 40 Kd.

[0043] In still further alternative aspects, it should be appreciated that the PEGylation may be mixed with respect to the attachment position of the PEG moiety and/or kind of attachment. Therefore, IL-11 may be subjected to random non-covalent PEGylation and site specific PEGylation at an N-terminal amino acid, or subjected to different site specific PEGylations at

the N-terminal amino acid and an internal amino acid. For example, and most preferably, IL-11 (or any modified form thereof) may be PEGylated at the N-terminal amino acid and optionally at an internal amino acid via a nitrogen atom (*e.g.*, from lysine or histidine) in addition to the N-terminal modification.

[0044] For example, and using the truncated IL-11 as shown Figure 1, the inventors performed PEGylation using the PEG reagents as shown in **Table 1** (where n and m are independently an integer between 80 and 1000, depending on the molecular weight of the compound) following experimental protocol as provided by the manufacturer and as described in more detail further below.

Manufacturer/ Cat. No.	Structure / Molecular size
NOF/ SUNBRIGHT ME-200AL	 20 KD
Jenkem/ Y-PLAD-40K	 40 KD
NOF/ SUNBRIGHT ME-120TS	 12 KD
NOF/ SUNBRIGHT GL2-400TS	 40 KD
NOF/ SUNBRIGHT GL2-200AL3	 20 KD
NOF/ SUNBRIGHT ME-050TS	 5 KD

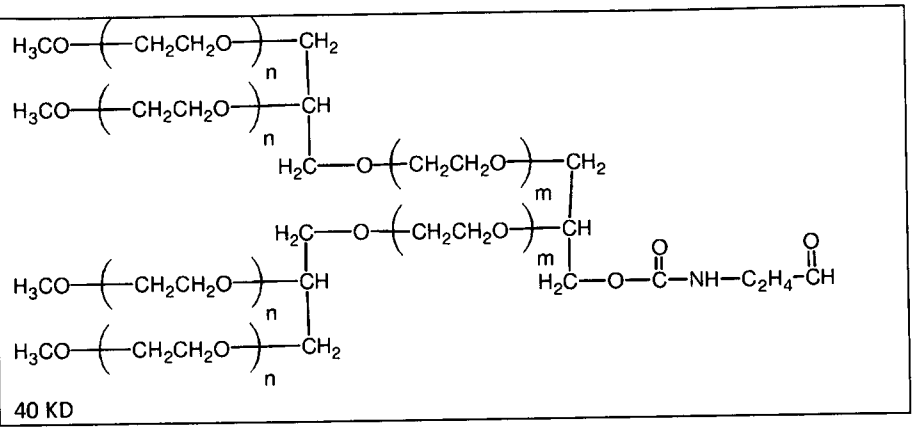
NOF/ SUNBRIGHT GL4-400AL3	 40 KD

Table 1

After PEGylation of the truncated IL-11, the so obtained compounds were purified as also addressed in more detail below, and the various PEGylated IL-11 molecules had the following designations as shown in Table 2:

Compound code	PEG size/KDa	Structure of PEG	Conjugation site (bonding)
I20NL	Single 20	linear	N-terminal (amine bond)
I40NY	Single 40	Y-shaped	N-terminal (amine bond)
I12KL	Single 12	linear	N-terminal (amide bond)
I40KY	Single 40	Y-shaped	N-terminal (amide bond)
I20NY	Single 20	Y-shaped	N-terminal (amine bond)
I20NL2	20 x 1~3	linear	N-terminal/lysine or histidine (amine bond)
I20NY2	20 x 1~3	Y-shaped	N-terminal/lysine or histidine (amine bond)
I05KL4	5 x 4~5	Linear	N-terminal/lysine/histidine (amide bond)
I40NX	Single 40	4-arm	N-terminal (amine bond)

Table 2

[0045] Most notably, the inventors have discovered that the type of PEG moiety and the site of attachment (and to some degree the sequence of the IL-11) had unexpected and substantial influence on biological activity and stability *in vivo*. As is more evident from the experimental data below, especially preferred PEGylations are at the N-terminal amino acid using a single Y-shaped PEG moiety, particularly where the IL-11 was truncated.

Contemplated Compositions

[0046] Based on the inventors' discovery of extended biological activity of contemplated compounds, it is generally contemplated that the compounds according to the inventive subject matter may be formulated for treatment of various diseases associated with a lack of IL-11 or characterized by a therapeutic response to treatment with IL-11. Therefore, and among other contemplated uses, the inventors especially contemplate that pharmaceutical compositions comprising contemplated compounds may be effective for the treatment or prevention of (a)

chemotherapy-induced thrombocytopenia, (b) nuclear accident/radiation induced bone and gastrointestinal (GI) damage; (c) chemotherapy induced bone and GI damage; (d) burn induced thrombocytopenia and GI damage; (e) other causes of thrombocytopenia; (f) other causes of GI damage, including inflammatory bowel diseases like Crohn's Disease and ulcerative colitis, as well as pseudomembraneous colitis, (g) free radical-induced lung damage, and/or (h) cardiovascular diseases, wherein contemplated pharmaceutical compositions comprise a therapeutically effective amount of contemplated compounds (or pharmaceutically acceptable salt, hydrate, or prodrug thereof), and a pharmaceutically acceptable carrier. For example, in one aspect of the inventive subject matter, contemplated compositions are formulated for treatment of chemotherapy-induced thrombocytopenia or GI damage or radiation induced bone and gastrointestinal (GI) damage. Alternatively, or additionally, it should also be appreciated that contemplated compositions may be formulated to induce acute phase proteins, and/or to modulate antigen-antibody responses.

[0047] It is particularly preferred that contemplated compounds are included in a composition that is formulated with one or more non-toxic pharmaceutically acceptable carriers. Suitable pharmaceutical compositions are preferably formulated for injection or infusion, or for oral administration in solid or liquid form. Thus, it should be appreciated that pharmaceutical compositions according to the inventive subject matter may be administered to humans and other (typically mammalian) animals using various routes, including parenterally, orally, intraperitoneally, and topically.

[0048] For example, suitable pharmaceutical compositions for injection preferably comprise pharmaceutically acceptable sterile aqueous or nonaqueous solutions, dispersions, emulsions, or suspensions, as well as sterile powders for reconstitution into sterile injectable solutions or dispersions prior to use. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents, or vehicles include water, Ringer's solution, and isotonic sodium chloride solution, ethanol, polyols (*e.g.*, glycerol, propylene glycol, polyethylene glycol, etc.), and suitable mixtures thereof, oils, and injectable organic esters (*e.g.*, ethyl oleate). Contemplated compositions may also contain various inactive ingredients, including preservatives, wetting agents, emulsifying agents, and/or dispersing agents. Sterility may be ensured by inclusion of antibacterial and/or antifungal agents (*e.g.*, paraben, phenol sorbic acid, chlorobutanol, etc.), as well as by filtration across sub-micron membranes (*e.g.*, 0.45 μM or 0.22 μM pore size), autoclaving or pasteurizing, and radiation (*e.g.*, gamma or e-beam). Where appropriate,

osmotically active agents may be included (*e.g.*, sugars, sodium chloride, etc.). While not limiting to the inventive subject matter, contemplated formulations for injection are typically in a pH range of 3-9, more typically 6-8, and most typically 7.4 +/- 0.3. Of course, it should also be recognized that all liquid formulations may be preserved in various manners to facilitate long-term storage/stockpiling. For example, contemplated manners of stabilization include water/solvent removal using lyophilization, spray-drying, crystallization, adsorption on (preferably biocompatible or pharmaceutically acceptable) solid phases, etc.

[0049] The compositions according to the inventive subject matter may be administered using various routes, including orally, parenterally, by inhalation, topically, rectally, nasally, or via an implanted reservoir, wherein the term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intraarticular, intrasynovial, intrathecal, intrahepatic, intralesional, and intracranial administration (typically injection or infusion). Preferably, the compositions are administered via injection, typically intravenously, and more preferably subcutaneously. Contemplated pharmaceutical compositions may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, including diseases of the eye, the skin, the lower intestinal tract, or areas exposed during surgical intervention. There are numerous topical formulations known in the art, and all of such formulations are deemed suitable for use herein.

[0050] With respect to the amount of contemplated compounds in the composition, it should be recognized that the particular quantity will typically depend on the specific formulation and desired purpose. Therefore, it should be recognized that the amount of contemplated compounds will vary significantly. However, it is generally preferred that the compounds are present in a minimum amount effective to deliver a therapeutic effect *in vitro* and/or *in vivo*.

[0051] Thus, in most preferred embodiments, contemplated compounds will be present in an amount of between about 0.1 µg/ml to about 100 mg/ml, more typically in an amount of between about 10 µg/ml to about 10 mg/ml, and most typically between about 5 µg/ml to about 100 µg/ml. With respect to a dosage unit, it is generally contemplated that contemplated compounds are administered at a dosage effective to achieve a desired therapeutic effect, typically 10-100 µg/kg, and more preferably 30-70 µg/kg. However, alternate dosage units may be between 0.1-10 µg/kg, or 50-80 µg/kg, or 80-120 µg/kg, or 120-200 µg/kg, or even higher. Viewed from a different perspective, it should be appreciated that a single-use unit of contemplated formulations may include between about 0.3 mg to 3.0 mg of PEGylated IL-11,

or between about 3 mg to 7 mg of PEGylated IL-11, or between about 7 mg to 10 mg of PEGylated IL-11 (most typically with a specific activity of 7-9 x10^6 U/mg). Unless the context dictates the contrary, all ranges set forth herein should be interpreted as being inclusive of their endpoints, and open-ended ranges should be interpreted to include commercially practical values. Similarly, all lists of values should be considered as inclusive of intermediate values unless the context indicates the contrary.

[0052] In addition, it should be noted that contemplated formulations may include one or more additional pharmaceutically active agents, which may be present in the same formulation, or be separately made available (in a different type of formulation or the same), or be sold as a kit. For example, suitable additional pharmaceutically active agents include various steroids (*e.g.*, corticosteroids), agents that stimulate platelet production in bone marrow (*e.g.*, Li_2CO_3, folic acid, etc.), antibodies, analgesics, and anti-inflammatory agents.

Contemplated Uses

[0053] Contemplated compounds may be particularly useful as therapeutic agents for single or combination use in the treatment of (a) nuclear accident/radiation induced bone and gastrointestinal (GI) damage; (b) chemotherapy induced bone and GI damage; (c) burn induced thrombocytopenia and GI damage; (d) other causes of thrombocytopenia; (e) other causes of GI damage, including inflammatory bowel diseases like Crohn's Disease and ulcerative colitis, as well as pseudomembraneous colitis, (f) free radical-induced lung damage, and (g) cardiovascular diseases.

[0054] Consequently, the inventors also contemplate use of the compounds presented herein for the manufacture of a drug for treatment of (a) nuclear accident/radiation induced bone and GI damage; (b) chemotherapy induced bone and GI damage; (c) burn induced thrombocytopenia and GI damage; (d) other causes of thrombocytopenia; (e) other causes of GI damage, including inflammatory bowel diseases like Crohn's Disease and ulcerative colitis, as well as pseudomembraneous colitis, (f) free radical-induced lung damage, and (g) cardiovascular diseases.

[0055] Viewed from another perspective, the inventors also contemplate methods of treatment of (a) nuclear accident/radiation induced bone and GI damage; (b) chemotherapy induced bone and GI damage; (c) burn induced thrombocytopenia and GI damage; (d) other causes of thrombocytopenia; (e) other causes of GI damage, including inflammatory bowel diseases like

Crohn's Disease and ulcerative colitis, as well as pseudomembraneous colitis, (f) free radical-induced lung damage, and (g) cardiovascular diseases in a human in need thereof in which contemplated compounds are administered at a therapeutically effective dosage.

Experiments and Experimental Data

[0056] Materials: Purified bulk of recombinant human IL-11, derived from yeast was provided by Hangzhou Jiuyuan Gene Engineering Company (Lot# 20121005/1006/1007/1008). 7TD1 murine hybridoma cell line, was acquired from DSMZ (No. ACC 23). Paraplatin® injection (generic name: carboplatin) 10 mg/mL (Lot: 5A03935) was manufactured by Bristol-Myers Squibb Company. Trypsin of sequencing grade, modified from bovine pancreas (Cat. No. 11418025001) was purchased from Roche diagnostics. Mouse IL-11 receptor alpha was acquired from MyBioSource, Inc. (Cat. No. MBS553276). CellTiter 96® Aqueous Non-Radioactive Cell Proliferation Assay (MTS) (Cat. No. G5430) was purchased from Promega for 7TD1 cell assay. The DuoSet ELISA development kit for Human IL-11 was purchased from R&D Systems Inc. (Cat. No. DY218). The Purification resin-MacroCap SP (Product code 17-5440-01) was acquired from GE Healthcare Life Sciences. The Precise Tris-Glycine 8-16% polyacrylamide gels were purchased from Thermo Scientific. Trifluoroacetic acid (Cat. No. 302031) and acetonitril (Cat. No. 34967) for HPLC use were purcahsed from Sigma-Aldrich.

[0057] Monofunctional PEG of various forms with Cat. No. SUNBRIGHT® ME-120TS, ME-200AL, GL2-400TS, ME-050TS, GL4-400AL3, GL2-200AL3, were purchased from NOF Corporation, and Y-PALD-40K was purchased from Jenkem Technology USA. Molecular structures of the PEG reagents were shown above in Table 1.

[0058] Preparation of I12KL/I40KY/I05KL4: 5 mg/mL protein was introduced with mixture of 1 to 2-fold molar ratio of respective PEG reagent (NOF/SUNBRIGHT ME-120TS for I12KL; SUNBRIGHT GL2-400TS for I40KY) and 50 mM $NaHCO_3$ at pH about 8. I05KL4 (PEG reagent: NOF/SUNBRIGHT ME-120TS) was prepared with the same manner except the molar ratio of PEG to protein was added at 12-fold. The reaction mixture was incubated at room temperature for 2 hours, followed by quenching with 2 mM glycine. PEGylated product was isolated using chromatographic purification procedures as followed. The PEG molecule was linked to protein by amide bonding.

[0059] Preparation of I20NL/I40NY/I20NY/I20NL2/I20NY2/I40NX: 5 mg/mL protein was introduced with mixture of 1 to 2-fold molar ratio of respective PEG reagent (NOF/SUNBRIGHT ME-200AL for I20NL and I20NL2; Jenkem/Y-PLAD-40 for I40NY; NOF/SUNBRIGHT GL2-200AL3 for I20NY and I20NY2; NOF/SUNBRIGHT GL4-400AL3 for I40NX), 10 mM sodium cyanoborohydride and 50 mM NaH_2PO_4. For conjugating onto two sites, the molar ratio of PEG was added at 3.5 to 5.5-fold. The pH was adjusted to about 4.5-5.0. The reaction mixture was incubated at room temperature for 24 hours, followed by quenching with 2 mM glycine. The PEG molecule was linked to protein by a more stable amine bonding. PEGylated product was isolated using chromatographic purification as followed.

[0060] Chromatographic Purification: The pH of protein solution was adjusted to 4-5 with 1M acetic acid, followed by centrifugation or filtration to remove particulates. Four volumes of water was introduced. For conjugate containing PEG over 20 KDa: The protein solution was loaded onto a MacroCap SP column (1x6 cm) that was equilibrated with buffer A containing 20 mM sodium acetate pH 5. The protein was eluted with gradient- or step-elution of buffer B, containing 20 mM sodium acetate pH 5 and 1M NaCl. For conjugate containing PEG below 20 KDa: The protein solution was loaded onto a MacroCap SP column (1x6 cm) that was equilibrated with buffer A containing 20 mM sodium phosphate pH 7. The protein was eluted with gradient- or step-elution of buffer B, containing 20 mM sodium phosphate pH 7 and 1M NaCl. A typical final product as analyzed in a SDS PAGE gel can be seen in **Figure 2**. Here, the left lane was loaded with molecular weight markers, and various PEGylated forms of IL-11 were loaded into the remaining lanes. Note that I40NY ran at an apparent molecular weight of over 100 Kd, bigger than the estimated one of 60 Kd, which is likely due to the Y-shaped of its PEG moiety. In further particularly preferred aspects, purification of contemplated compounds is performed as a one-step purification process, which provides added advantages in a downstream scale-ups.

[0061] Purity Check by RP-HPLC: The content of each PEGamer was analyzed by reverse-phase (RP) chromatography employing the UPLC coupled with diode-array detector-UltiMate 3000 Rapid Separation LC Systems from Thermo Scientific. The chromatographic procedure was carried out using: Column: Acquity C18, 1.7 μm, 2.1 x 150 mm, 300 Å pore size, equipped with a guard cartridge; Mobile phase A: 0.1% (v/v) TFA in 50% (v/v) acetonitrile; Mobile phase B: 0.1% (v/v) TFA in 95% (v/v) acetonitrile; Flow rate: 0.4 ml/min;

Column temperature: 65°C; Detection: 214 nm; Inject 20 µg and run gradient as in Table 3 below

Time (min)	A%	B%
0	100	0
2	100	0
9.9	80	20
9.95	0	100
11.3	0	100
11.31	100	0
17.5	100	0

Table 3

[0062] Determination of Protein Content: The protein content was determined by the UV/Vis microplate and cuvette spectrophotometer- Multiskan GO from Thermo Scientific. Extinction coefficient in units of M^{-1} cm^{-1}, at 280 nm measured in water is 17,990. Alternatively protein concentration is directly determined by ultraviolet spectroscopy at wavelength 280 nm, using the absorbency value of 0.944 for a 0.1% (1 mg/ml) solution. Protein quantitation using absorbance at 280 nm measures the absorbance of aromatic amino acids such as tryptophan and tyrosine, leaving PEG moiety undetected. As a result, protein concentration by weight stated herein does not contain PEG molecule.

[0063] Pharmacokinetics (PK) study in healthy rats: The *in vivo* manipulation was carried out in 3 male Sprague-Dawley rats following single dose administration of contemplated compounds by intravenous or subcutaneous route at a dosing level of 100-150 µg/kg. Blood samples were collected in numerous time points in heparin tubes, followed by plasma separation and storage at -20°C. The concentration of immunoreactive IL-11 in plasma samples were determined by the DuoSet ELISA kit for Human IL-11 (R&D Systems Inc. Cat. No. DY218). The parameters of pharmacokinetics were yielded by the WinNonlin 5.3 software using non-compartment model.

[0064] Pharmacodynamics (PD) study in healthy rats: The pharmacodynamics assessment was carried out in 4 male Sprague-Dawley rats using intravenous or subcutaneous administration of respective contemplated compounds at dosing strength of 100-150 µg/kg. Blood samples were collected in numerous time points in heparin tubes, followed by plasma separation and storage at -20°C. The blood cell count was carried out on a Cell-DYN 3500 hematology analyzer.

[0065] Pharmacodynamics (PD) study in myelosuppressive rats: The pharmacodynamics assessment was carried out in 4 male Sprague-Dawley rats, using intravenous administration of carboplatin at 40 mg/kg on Day 0 to induce myelosuppression. Contemplated compounds were

subcutaneously injected on Day 1 at 150 μg/kg. Blood samples were collected in numerous time points in heparin tubes, followed by plasma separation and storage at -20°C. The blood cell count was carried out on a Cell-DYN 3500 hematology analyzer.

[0066] Tryptic mapping: The reaction solution was prepared in 50 mM Tris pH 8.3 buffer containing 2 mg/mL protein and 1/50 (W/W) trypsin. Incubated at room temperature for 6 hours, followed by adding equal volume of 0.2% TFA (trifluoroacetic acid) solution. Any particulate matters were removed by centrifuge prior injection onto HPLC. The chromatographic procedure was carried out using: Column: Zorbax 300 SB-C8, 2.1x150 mm, 5 μm, 300Å pore size; Mobile phase A: 0.1% (v/v) TFA; Mobile phase B: 0.1% (v/v) TFA in 95% (v/v) acetonitrile; Flow rate: 0.2 ml/min; Detection: 214 nm; Inject 10 μg and run gradient as in Table 4 below.

Time (min)	A%	B%
0	100	0
3	100	0
8	95	5
45	55	45
45.1	0	100
52	0	100
52.1	100	0
65	100	0

Table 4

The identification of proteolytic peptide was carried out with HPLC coupled with MS spectrometry (Thermo LCQ Advantage).

[0067] In some embodiments, the numbers expressing quantities of ingredients, properties such as concentration, reaction conditions, and so forth, used to describe and claim certain embodiments of the invention are to be understood as being modified in some instances by the term "about." Accordingly, in some embodiments, the numerical parameters set forth in the written description and attached claims are approximations that can vary depending upon the desired properties sought to be obtained by a particular embodiment. In some embodiments, the numerical parameters should be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of some embodiments of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as practicable. The numerical values presented in some embodiments of the invention may contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

[0068] While it is generally known that PEG can impart the plasma stability of its conjugates, it is not predictable what attachment type, chain length, and molecular structure will produce a specific result with respect to therapeutic effect and/or pharmacologic parameter. In a first example, the truncated IL-11 conjugates of various PEG size were investigated with regard to plasma stability in normal rats. After intravenous administration, the observed plasma half-life of un-PEGylated IL-11 was found to be very short, less than 10 minutes as compared to those (3.5-13.7 hr) of its PEGylated counterparts. Among the latter, higher molecular-weight PEG imparted greater plasma stability in the following order: I40KY (13.7hr) ~ I40NY (8.5hr) > I20NL (3.8hr) ~I12KL (3.5hr). **Figure 3** illustrates the plasma concentration of the various forms of PEGylated IL-11 after single intravenous administration. Each sample was dosed at 100 μg/kg in rats. It was concluded that in this example larger or longer PEG chain resulted in longer serum half-life.

[0069] In a second example, pharmacodynamics assessment was carried out with healthy Sprague-Dawley rats following intravenous route measuring platelet increment after single intravenous administration at 100 μg/kg. As can be seen, from **Figure 4**, 40-KD conjugates (I40NY and I40KY) induced more platelet increment (60-75%) than I20NL (50%), where Y represented the Y-shape PEG and L the linear shape PEG. The results also suggested multiple conjugation (I05KL4, 5-KD PEG conjugated on four sites) to be less effective than a single long PEG chain on the N-terminus, as multiples with shorter PEG conjugates had only limited efficacy at about 25% of platelet increment. It was concluded in this example that longer PEG chain on single site resulted in a higher efficacy in the respect of platelet induction. This appears to be opposite to the effect of PEGylation on recombinant human growth hormone.

[0070] In a third example, conjugation sites were investigated by tryptic mapping, coupled with LC/MS identification of proteolytic peptides. **Figure 5** depicts tryptic maps of IL-11 (unconjugated), I40NY, and I40KY, and Table 5 below provides the tryptic peptides.

Mass	Position	Peptide #	Peptide Sequence
773.9	1-8	T1	GPPPGPPR
669.7	9-14	T2	VSPDPR
1217.4	15-25	T3	AELDSTVLLTR
774.9	26-32	T4	SLLADTR
798.9	33-39	T5	QLAAQLR
261.3	40-41	T6	DK
3319.8	42-74	T7	FPADGDHNLDSLPTLAMSAG ALGALQLPGVLTR
287.4	75-76	T8	LR
950.1	77-84	T9	ADLLSYLR
839.0	85-90	T10	HVQWLR

174.2	91-91	T11	R
618. 7	92-98	T12	AGGSSLK
1327.5	99-110	T13	TLEPELGTLQAR
402.5	111-113	T14	LDR
400.5	114-116	T15	LLR
174.2	117-117	T16	R
860.1	118-124	T17	LQLLMSR
2600.0	125-150	T18	LALPQPPPDPPAPPLAPPSS AWGGIR
1914.2	151-168	T19	AAHAILGGLHLTLDWAVR
655.9	169-174	T20	GLLLLK
275.3	175-176	T21	TR
131.2	177-177	T22	L

Table 5

[0071] Here it can be seen that the peak corresponding to the T1 peptide was noticeably reduced in the tryptic maps of I40NY and I40KY. This indicates that both conjugates with PEG were linked onto T1 peptides, where the N-terminal amine was the only site for the chemical conjugation. As a result, I40NY and I40KY were both N-terminally linked, however only differed in chemical bonding with amine bond for I40NY and amide bond for I40KY. Notably, both I40NY and I40KY exhibited similar effects in serum half-life and platelet induction via intravenous administration.

[0072] Since an amine bond is more stable than an amide bond, and yield of mono-PEGylated product was more homogeneous for selective PEGylation using reductive amination, in the next study, various N-terminal conjugates were investigated in terms of efficacy in platelet production, and were evaluated by their respective associated side-effect via subcutaneous administration in healthy rats. **Figure 6** depicts results of a pharmacodynamics study of six PEGylated IL-11 conjugates in rats with subcutaneous administration at 150 μg/kg. IL-11 was administered daily for continuous 14 days while PEGylated IL-11 were injected once weekly. PEG shape can influence the function of the conjugate. Particularly, the non-linear shape PEG molecule imparts better plasma stability and more potency than its linear counterpart. As displayed in Figure 6, I20NY induced more platelet increment (58-70%) than I20NL (46-55%), where Y represented the Y-shape PEG and L the linear shape PEG. These results suggested that a Y-shaped PEG had a greater effectiveness than the linear form of the same molecular weight. However, I40NY (Y-shape) and I40NX (4-arm comb-shape) were comparable in platelet production as both increased up to about 65-70%, suggesting the influence of PEG shape became saturated when the PEG size was about or over 40KD. Notably, double PEGylation of the same PEG length reduced the *in vivo* efficacy, as I20NL2 (linear PEG onto two sites) and I20NY2 (Y-shaped PEG onto two sites) had lower platelet production than their single PEGylated counterparts. It was therefore concluded that I40NY,

I40NX and I20NY exerted higher efficacy among various N-terminal conjugated IL-11s. Moreover, it was noted that the effect of second administration was somehow down-regulated for smaller PEG conjugates such as 20-KD PEG regardless of number of conjugation site. As a result, I40NY and I40NX were unexpectedly effective compounds with desirable biological properties and relatively moderate adverse effects (especially plasma expansion). Moreover, the biological data further suggest that the so modified IL-11 compounds can be administered less frequently, and most preferably twice weekly, once weekly, or even less. Such schedule is particularly relevant where contemplated compounds are employed in the treatment of thrombocytopenia in a large population (*e.g.*, exposed to radiologic exposure).

[0073] The inventors also studied the side-effect associated with IL-11 conjugates in healthy rats. Hematocrit status is usually used as a marker for assessment of side-effect in clinical use of IL-11, because patients might experience dilutional anemia due to plasma expansion. In the animal study, IL-11 was administered subcutaneously for continuous 14 days at 150 μg/kg while PEGylated IL-11 were injected once weekly at the same dose. As shown in **Figure 7**, all drugs resulted in decreased hematocrit but I40NY was found to have less reduction while maintained higher activity than the rest of PEGylated conjugates. The mitigated dilutional anemia of dosing with I40NY is more prominent when compared in the same chart with other individual animal experiments using conjugated and unconjugated IL-11s. A correlation between platelet production and side-effect as manifested by reduction of hematocrit was established in **Figure 8**, suggesting a trend of intensifying side-effect along with the increasing and dose-dependent efficacy when various modified and unmodified IL-11s were plotted in the chart. I40NYs, at different doses, were distinctly located on the right upper side of the trend, indicating less plasma expansion than some other compounds and unmodified IL-11 on the basis of comparable efficacy. In terms of product characterization, the inventors characterized physicochemical and pharmacological properties of a preferred compound, I40NY.

[0074] Cell-based Assay for IL-11 Conjugates: The biological activities of conjugated IL-11 were tested in a cell proliferation assay, using 7TD1 cell-line (DSMZ, Germany). In brief, 7TD1 cells at 4,000 cells per well were grown at 37°C in a humidified atmosphere of 5% CO_2 in response to different IL-11 concentrations for two days in the presence of 2 μg/mL murine IL-11 receptor (MyBioSource, USA, MBS553276)(*Biochem.J., 318:489-495*). After addition of MTS, the EC50 of dose response curve was determined by plotting the absorbance of 490 nm on the y-axis against IL-11 concentrations on the y-axis, by fitting sigmoid dose-response

curves with GraphPad software Prism 6. Before animal studies, the biological activities of newly synthesized conjugates were tested in a cell proliferation assay, using 7TD1 cell line. Not all PEGylation preparations gave similar products, and the actual product formation depends on the amino acid residues of the IL-11 being conjugated, and on the size and the shape of the PEG molecule used. 7TD1 cells grew in response to different conjugate concentrations. After addition of developers, whose chemical signal has a linear relationship with the cell number, absorbance at 490 nm was read in an ELISA plate-reader. The results were shown in **Figure 9**.

[0075] Due to the steric hindrance of the PEG moiety, all the conjugates expectedly showed a reduction in bioactivity in the cell-based assay as compared to that of the un-PEGylated IL-11 with the following order of potency: IL-11 (100%) > I20NL, I20NY (both about 16%) > I40NY, I40NX (both 11%) > I40KY (6%) >I20NY2 (3%). Notably, the steric interference is a dominant factor in determining biological activity of PEGylated conjugates and can be seen as a drastic decrease in bioactivity of the conjugate whose overall PEG moiety is bigger than 20 KDa. From some cell-based studies, there was a report showing that a small carbohydrate attachment at the non-core region of the IL-11 molecule, such as N-terminal sequence, enhanced the biological activity when compared with those conjugates at other attachment sites (*J. Biol. Chem. Vol 286, No. 10, pp 8085-8093*), which was consistent with less reduction of bioactivity by PEG molecule at the N-terminal sequence of IL-11. Although the *in vitro* bioactivity of I40NY retained only about 11% of native IL-11, *in vivo* efficacy was beneficially affected and could not be predicted from the *in vitro* bioactivity data. Table 6 below schematically illustrates bioactivity ratios of various compounds relative to unmodified IL-11.

Identification	Bioactivity ratio to unmodified IL-11
IL-11	1
I20NL	0.13
I20NY	0.16
I20NY2	0.03
I40NY	0.11
I40NX	0.11
I40KY	0.06

Table 6

[0076] Chemical modification of proteins with PEG is an established technology and has been applied to biopharmaceutical industry to enhance the solubility and physical-chemical stability of proteins. While this chemical reaction is easy to carry out, it often results in a complex mixtures of different PEGylated forms, containing PEGamer and positional isomers. Multiple chromatographic purifications steps are employed to isolate product with high recovery. To

develop a commercially viable process in terms of cost and yield, many factors such as protein concentration, quality of PEG, protein/PEG ratio, reaction temperature, and buffer pH, as well as purification process, are required to be optimized.

[0077] I40NY was constructed by conjugation with a Y-shaped polyethylene glycol chain on amines forming stable amine bond, with relatively high selectivity to the N-terminal amine driven by conjugation chemistry (reductive amination of aldehyde coupling group in PEG moiety). I40KY on the other hand was conjugated using a functionalized NHS reagent at pH 8 on accessible amines forming the corresponding amide bonds . More specifically, I40NY is the mono-PEGylated IL-11 produced with site-specific reaction under acidic conditions because functionalized aldehyde is largely selective for the N-terminal α-amine, whose pKa is lower compared to other nucleophiles. PEG to protein ratio, reaction concentration, pH and kinetics were investigated in the conjugation reaction. Reactions were taken place for 24 hours at room temperature (22-27°C) in small scale at about 0.05-0.5 mL in volume in the presence of 10 mM sodium cyanoborohydride. Yield of each reaction under investigation was determined by RP-UPLC. Using different pH for selected reactions, optimum conjugation yield was at pH 4.5-5.5. In addition, the inventors noted that the concentration of reactants played an important factor in product yield and discovered that conjugation with IL-11 at concentrations larger than 5 mg/mL were optimal. Likewise, the PEG to protein ratio and conjugation kinetics of the reaction with 5 mg/mL protein at room temperature in the presence of reducing agent were investigated and suggested an optimum molar ratio of 2 for PEG to protein, and reaction extension to 16 hours sufficient for mono-PEGylation.

[0078] Purification of PEGylated protein usually employs ion-exchange chromatography in large-scale preparation. However a satisfactory resolution to separate the mono-PEGylated from the oligo-PEGylated is not achievable when conventional ion-exchanger is loaded with reaction product at a loading capacity as little as 1 mg/mL resin. The low capacity of this resin often limits its application for larger scale production. To isolate N-terminally mono-PEGylated IL-11 at high purity, various cation-exchange resins were tested. Notably, high porosity resins (e.g., MacroCap SP from GE Healthcare Life Sciences) provided high capacity with retained resolution, and offered high purity and yield of mono-PEGylated targets at high load conditions. The purification process was demonstrated with a batch size of 400 mg IL-11, prepared in 5 mg/mL in sodium phosphate pH 4.5-5 buffer containing 2 molar ratio of aldehyde-activated 40-KD Y-shaped PEG reagent in the presence of 10 mM sodium

cyanoborohydride. The reaction solution was quenched by adding 2 M glycine followed by dilution with 4X volume of deionized water. After filtration through a 0.2 μm membrane, the resulting crude was loaded onto a MacroCap SP column (2.6 (diameter) x 10 (height) cm at a loading capacity of about 7.5 mg/mL resin). After charging, the column was washed with 20 mM sodium acetate pH 5 buffer over 10 column volume, followed by additional wash with 20 mM sodium acetate pH 5 buffer containing 0.1 M NaCl over 20 column volume. The product was then eluted with 20 mM sodium acetate pH 5 buffer containing 0.3 M NaCl. The overall yield of isolating I40NY was 26.6%. The product purity of I40NY was examined by SDS-PAGE and reverse-phase HPLC, and **Figure 10** shows purity of I40NY on a silver-stained SDS-PAGE gel with quantities of I40NY as indicated above the lanes. The purity of mono-PEGylated IL-11 was larger than 93% as determined by C18-HPLC as the chromatogram displayed in **Figure 11**.

[0079] To determine the pharmacokinetic parameters of I40NY via subcutaneous route, 3 male Sprague-Dawley rats were injected with 0.15 mg/kg of PEGylated IL-11 via single subcutaneous administration. **Figure 12** displayed the plasma concentration of immunoreactive IL-11 in rats after single subcutaneous administration. Plasma concentration of conjugated IL-11 achieved a maximal level at about 12 hr and remained effective over 50 hr after administration. On the contrary, the recombinant human IL-11 reached a maximal concentration at about 2 hr, and was cleared from the circulation blood stream rapidly as the elimination half-life in plasma was about 1.3 hr. The pharmacokinetic parameters of I40NY via subcutaneous route were summarized in Table 7 below.

	unit	I40NY	IL-11
$T_{1/2}$, terminal half-life	hr	18.6	1.1
T_{max}, time to maximal concentration	hr	12	2
C_{max}, maximal plasma concentration	ng/mL	142	147
AUC_{all}, area under curve	Hr*ng/mL	3947	700
AUC_{inf}, area under curve to infinity	Hr*ng/mL	4421	701
Vz, relative volume of distribution	mL/kg	909	347
Cl, relative clearance	mL/hr/Kg	33.9	214
MRT, mean residence time	hr	27.2	3.7

Table 7

[0080] The secondary structure of I40NY was investigated using circular dichroism. In the chromatogram of a circular dichroism analyzed in the far-UV region, the inventors demonstrated that I40NY maintained the same secondary structure as its unconjugated counterpart, as can be seen from both spectra superimposed in **Figure 13.** Moreover the thermal stability of I40NY was demonstrated by circular dichroism by measuring the change of

their secondary structures (mean residue ellipticity) in response to thermal stress. **Figure 14** indicated the structural change being less for I40NY in response to temperature increment.

[0081] The effectiveness of I40NY in myelosuppressive rats was also demonstrated in carboplatin-treated rats. Male Sprague-Dawley rats were injected with 40 mg/kg of carboplatin via intravenous administration to induce damaged function of bone marrow leading to thrombocytopenia. Medical intervention using daily injection (consecutive 7 days) of IL-11 or single dose of I40NY at the same 0.15 mg/kg dosage was subcutaneously administered immediately after 24 hours of carboplatin treatment. The platelet level was displayed in **Figure 15.** Without treatment, subjects experienced about two days of severe thrombocytopenia (less than 1/3 of normal platelet count), suggesting the untreated having a high risk of life threatening internal bleeding. The efficacy of IL-11 treatment was marginal as the nadir of daily dosing was very close to the threshold of severe thrombocytopenia. Single dose of I40NY, on the contrary, not only prevented the occurrence of severe thrombocytopenia but also accelerated the recovery of platelet levels, as the platelet count returning to the initial number was 1.3 days earlier than the other two groups.

[0082] Meanwhile, the side effects as manifested on the reduction of hematocrit was also investigated in the myelosuppressive model. In **Figure 16**, treating with IL-11s caused hematocrit reduction in a rapid manner compared to the untreated group. However single dose of I40NY alleviated the nadir, suggesting less intensified side-effect than daily dosing with IL-11. Thus, it should be appreciated that I40NY has proven effective in preventing severe thrombocytopenia induced by chemotherapy, while ameliorating the syndrome of plasma expansion.

[0083] Further comparative data between I40NY and another form of a PEGylated IL-11 (as described in US8133480, data not shown) reveal that contemplated compounds, and especially I20NY and I40NY had significantly enhanced *in vivo* effectiveness and reduced syndrome of side-effect as compared to the other form of PEGylated IL-11 as described in the '480 patent.

[0084] It should be apparent to those skilled in the art that many more modifications besides those already described are possible without departing from the inventive concepts herein. The inventive subject matter, therefore, is not to be restricted except in the scope of the appended claims. Moreover, in interpreting both the specification and the claims, all terms should be interpreted in the broadest possible manner consistent with the context. In particular, the terms

"comprises" and "comprising" should be interpreted as referring to elements, components, or steps in a non-exclusive manner, indicating that the referenced elements, components, or steps may be present, or utilized, or combined with other elements, components, or steps that are not expressly referenced. Where the specification claims refers to at least one of something selected from the group consisting of A, B, C .... and N, the text should be interpreted as requiring only one element from the group, not A plus N, or B plus N, etc.

SEQUENCE LISTING

<110> Nansha Biologics (Hong Kong)

<120> COMPOSITIONS AND METHODS FOR PEGYLATED IL-11

<130> 102635.0001PCT

<150> US 62/127,748
<151> 2015-03-03

<160> 1

<170> PatentIn version 3.5

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<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
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<223> human IL-11 lacking N-terminal P

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20 25 30

Gln Leu Ala Ala Gln Leu Arg Asp Lys Phe Pro Ala Asp Gly Asp His
35 40 45

Asn Leu Asp Ser Leu Pro Thr Leu Ala Met Ser Ala Gly Ala Leu Gly
50 55 60

Ala Leu Gln Leu Pro Gly Val Leu Thr Arg Leu Arg Ala Asp Leu Leu
65 70 75 80

Ser Tyr Leu Arg His Val Gln Trp Leu Arg Arg Ala Gly Gly Ser Ser
85 90 95

Leu Lys Thr Leu Glu Pro Glu Leu Gly Thr Leu Gln Ala Arg Leu Asp
100 105 110

Arg Leu Leu Arg Arg Leu Gln Leu Leu Met Ser Arg Leu Ala Leu Pro
115 120 125

Gln Pro Pro Pro Asp Pro Pro Ala Pro Pro Leu Ala Pro Pro Ser Ser
130 135 140

Ala Trp Gly Gly Ile Arg Ala Ala His Ala Ile Leu Gly Gly Leu His
145 150 155 160

Leu Thr Leu Asp Trp Ala Val Arg Gly Leu Leu Leu Leu Lys Thr Arg
165 170 175

Leu

CLAIMS

What is claimed is:

1. A modified interleukin 11 (IL-11) compound, comprising:
   an IL-11 polypeptide chain covalently coupled to a PEG moiety, wherein the PEG moiety has an average molecular weight of between 10-50Kd and has distinct first and second PEG portions;
   wherein the PEG moiety is covalently bound to an N-terminal amino acid; and
   wherein the IL-11 polypeptide chain is a human or humanized polypeptide chain.

2. The modified compound of claim 1 wherein the IL-11 polypeptide chain is a human IL-11 polypeptide chain.

3. The modified compound of any one of claims 1 or 2 wherein the IL-11 polypeptide chain is shortened by deletion of an N-terminal proline.

4. The modified compound of any one of the preceding claims wherein the IL-11 polypeptide chain has a sequence according to SEQ ID NO:1.

5. The modified compound of any one of the preceding claims wherein the PEG moiety has an average molecular weight of 20Kd or 40Kd.

6. The modified compound of any one of the preceding claims wherein the PEG moiety has a Y shape.

7. The modified compound of any one of the preceding claims wherein a molar ratio of polypeptide chain to PEG moiety is about 1:1.

8. The modified compound of any one of the preceding claims further comprising a second PEG moiety covalently coupled via an amino group of the IL-11 polypeptide chain.

9. The modified compound of any one of the preceding claims wherein the PEG moiety is covalently bound to the N-terminal amino acid via an amine bond.

10. A pharmaceutical composition comprising a therapeutically effective amount of an IL-11 compound according to any one of claims 1-9, in combination with a pharmaceutically acceptable carrier.

11. The pharmaceutical composition of claim 10 wherein the composition is formulated for injection.

12. The pharmaceutical composition of claim 10 wherein the IL-11 compound is present in an amount to provide a dosage unit of between 10-100 μg/kg for a pediatric or adult patient.

13. The pharmaceutical composition of claim 10 wherein the composition is lyophilized.

14. The pharmaceutical composition of claim 10 further comprising a second pharmaceutically active compound.

15. The pharmaceutical composition of claim 14 wherein the second pharmaceutically active compound is a steroid, an agent that stimulates platelet production in bone marrow, an antibody, an analgesic, or anti-inflammatory agent.

16. Use of an IL-11 compound according to any one of claims 1-9 in the manufacture of a pharmaceutical composition.

17. The use of claim 16 wherein the pharmaceutical composition is for treatment of a condition selected from the group consisting of (a) nuclear accident/radiation induced bone and gastrointestinal damage; (b) chemotherapy induced bone and gastrointestinal damage; (c) burn induced thrombocytopenia and gastrointestinal damage; (d) chemotherapy induced thrombocytopenia; (e) trauma-, cancer-, or infection-induced gastrointestinal damage or inflammatory bowel disease, (f) free radical-induced lung damage, and (g) a cardiovascular disease.

18. The use of claim 16 wherein the pharmaceutical composition is formulated for injection.

19. The use of claim 16 wherein the pharmaceutical composition is lyophilized.

20. A method of increasing serum half-life of an interleukin 11 (IL-11) compound, comprising:

covalently coupling an IL-11 polypeptide chain to a PEG moiety, wherein the PEG moiety has an average molecular weight of between 10-50Kd and has distinct first and second PEG portions;

wherein the PEG moiety is covalently bound to an N-terminal amino acid; and

wherein the IL-11 polypeptide chain is a human or humanized polypeptide chain.

21. The method of claim 20 wherein the IL-11 polypeptide chain is a human IL-11 polypeptide chain.

22. The method of any one of claims 20-21 wherein the IL-11 polypeptide chain is shortened by deletion of an N-terminal proline.

23. The method of any one of claims 20-22 wherein the IL-11 polypeptide chain has a sequence according to SEQ ID NO:1.

24. The method of any one of claims 20-23 wherein the PEG moiety has an average molecular weight of 20Kd or 40Kd.

25. The method of any one of claims 20-24 wherein the PEG moiety has a Y shape.

26. The method of any one of claims 20-25 wherein a molar ratio of polypeptide chain to PEG moiety is about 1:1.

27. The method of any one of claims 20-26 further comprising a step of covalently coupling a second PEG moiety via an amino group in the IL-11 polypeptide chain.

28. The method of any one of claims 20-27 wherein the PEG moiety is covalently bound to the N-terminal amino acid via an amine bond.

29. A method of treating a condition responsive to administration of IL-11, comprising a step of administering the pharmaceutical composition of claim 10 in a therapeutically effective amount to a patient in need thereof.

30. The method of claim 29 wherein the condition is selected from the group consisting of (a) nuclear accident/radiation induced bone and gastrointestinal damage; (b) chemotherapy induced bone and gastrointestinal damage; (c) burn induced thrombocytopenia and gastrointestinal damage; (d) chemotherapy induced thrombocytopenia; (e) trauma-, cancer-, or infection-induced gastrointestinal damage or inflammatory bowel disease, (f) free radical-induced lung damage, and (g) a cardiovascular disease.

31. The method of claim 29 or 30 wherein the pharmaceutical composition comprises IL-11 I40NY or I20NY.

32. The method of any one of claims 29-31 wherein the IL-11 is administered in a dosage between 10-100 μg/kg.

33. The method of any one of claims 29-32 wherein the IL-11 is administered subcutaneously.

AMENDED CLAIMS
received by the International Bureau on 30 July 2016 (30.07.2016)

CLAIMS

What is claimed is:

1. A modified interleukin 11 (IL-11) compound, comprising:
   an IL-11 polypeptide chain covalently coupled to a PEG moiety, wherein the PEG moiety has an average molecular weight of between 10-50Kd and has distinct first and second PEG portions;
   wherein the PEG moiety is covalently bound to an N-terminal amino acid and the molar ratio of IL-11 polypeptide chain to PEG moiety ranges from 1:1 to 0.8:1; and
   wherein the IL-11 polypeptide chain is a human or humanized polypeptide chain.

2. The modified compound of claim 1 wherein the IL-11 polypeptide chain is a human IL-11 polypeptide chain.

3. The modified compound of any one of claims 1 or 2 wherein the IL-11 polypeptide chain is shortened by deletion of an N-terminal proline.

4. The modified compound of claim 1 wherein the IL-11 polypeptide chain has a sequence according to SEQ ID NO:1.

5. The modified compound of claim 1 wherein the PEG moiety has an average molecular weight of 20Kd or 40Kd.

6. The modified compound of claim 1 wherein the PEG moiety has a Y shape.

7. The modified compound of claim 1 wherein a molar ratio of polypeptide chain to PEG moiety is about 1:1.

9. The modified compound of claim 1 wherein the PEG moiety is covalently bound to the N-terminal amino acid via an amine bond.

10. A pharmaceutical composition comprising a therapeutically effective amount of an IL-11 compound according to claim 1, in combination with a pharmaceutically acceptable carrier.

11. The pharmaceutical composition of claim 10 wherein the composition is formulated for injection.

12. The pharmaceutical composition of claim 10 wherein the IL-11 compound is present in an amount to provide a dosage unit of between 10-100 μg/kg for a pediatric or adult patient.

13. The pharmaceutical composition of claim 10 wherein the composition is lyophilized.

14. The pharmaceutical composition of claim 10 further comprising a second pharmaceutically active compound.

15. The pharmaceutical composition of claim 14 wherein the second pharmaceutically active compound is a steroid, an agent that stimulates platelet production in bone marrow, an antibody, an analgesic, or anti-inflammatory agent.

16. Use of an IL-11 compound according to claim 1 in the manufacture of a pharmaceutical composition.

17. The use of claim 16 wherein the pharmaceutical composition is for treatment of a condition selected from the group consisting of (a) nuclear accident/radiation induced bone and gastrointestinal damage; (b) chemotherapy induced bone and gastrointestinal damage; (c) burn induced thrombocytopenia and gastrointestinal damage; (d) chemotherapy induced thrombocytopenia; (e) trauma-, cancer-, or infection-induced gastrointestinal damage or inflammatory bowel disease, (f) free radical-induced lung damage, and (g) a cardiovascular disease.

18. The use of claim 16 wherein the pharmaceutical composition is formulated for injection.

19. The use of claim 16 wherein the pharmaceutical composition is lyophilized.

20. A method of increasing serum half-life of an interleukin 11 (IL-11) compound, comprising:

covalently coupling an IL-11 polypeptide chain to a PEG moiety, wherein the PEG moiety has an average molecular weight of between 10-50Kd and has distinct first and second PEG portions;

wherein the PEG moiety is covalently bound to an N-terminal amino acid, and wherein the molar ratio of IL-11 polypeptide chain to PEG moiety ranges from 1:1 to 0.8:1; and

wherein the IL-11 polypeptide chain is a human or humanized polypeptide chain.

21. The method of claim 20 wherein the IL-11 polypeptide chain is a human IL-11 polypeptide chain.

22. The method of any one of claims 20-21 wherein the IL-11 polypeptide chain is shortened by deletion of an N-terminal proline.

23. The method of claim 20 wherein the IL-11 polypeptide chain has a sequence according to SEQ ID NO:1.

24. The method of any claim 20 wherein the PEG moiety has an average molecular weight of 20Kd or 40Kd.

25. The method of claim 20 wherein the PEG moiety has a Y shape.

26. The method of claim 20 wherein a molar ratio of polypeptide chain to PEG moiety is about 1:1.

27. The method of claim 20 further comprising a step of covalently coupling a second PEG moiety via an amino group in the IL-11 polypeptide chain.

28. The method of claim 20 wherein the PEG moiety is covalently bound to the N-terminal amino acid via an amine bond.

29. A method of treating a condition responsive to administration of IL-11, comprising a step of administering the pharmaceutical composition of claim 10 in a therapeutically effective amount to a patient in need thereof.

30. The method of claim 29 wherein the condition is selected from the group consisting of (a) nuclear accident/radiation induced bone and gastrointestinal damage; (b) chemotherapy induced bone and gastrointestinal damage; (c) burn induced thrombocytopenia and gastrointestinal damage; (d) chemotherapy induced thrombocytopenia; (e) trauma-, cancer-, or infection-induced gastrointestinal damage or inflammatory bowel disease, (f) free radical-induced lung damage, and (g) a cardiovascular disease.

31. The method of claim 29 or 30 wherein the pharmaceutical composition comprises IL-11 I40NY or I20NY.

32. The method of claim 29 wherein the IL-11 is administered in a dosage between 10-100 µg/kg.

33. The method of claim 29 wherein the IL-11 is administered subcutaneously.

STATEMENT UNDER ARTICLE 19(1)

The Office considers claims 1 to 3 and claims 20 to 22 to be lack an inventive step over Japanese Patent Application JP2005-281302A, to Astellas Pharma, Inc. (hereafter referred to as D1). The Office states that D1 teaches a PEG modified IL-11, which can be recombinant and lack an N-terminal proline residue. The Office further states that D1 teaches that such PEG modified IL-11 includes 2 to 4 PEG per IL-11 (i.e. an IL-11 to PEG moiety molar ratio of 1:2 to 1:4), and states that the N-terminal position of the PEG moiety of the claims is a minor difference over the teachings of D1 that falls under the general knowledge of a person of ordinary skill. Claims 3 to 19 and 23 to 33 are unexamined due to either multiple dependencies or direction towards methods of treatment.

The Applicant notes that independent claims 1 and 20 have been amended to state that the IL-11 peptide to PEG moiety ratio is from 1:1 to 0.8:1, which is supported by at least paragraphs [0014] and [0042] of the instant application as submitted. Such a ratio indicates a high degree of selectivity for the N-terminal position that is neither taught or suggested by D1. The Applicant notes that a person of ordinary skill in the biochemical arts is aware that both the site and degree of substitution can have a profound effect on activity and stability in PEG-peptide conjugates. As such, teachings of D1 in regards the taught highly substituted and non-N terminally selective PEG modification of IL-11 cannot be inferred upon the claimed PEG-modified IL-11. The Applicant further notes that the degree of stability provided by this highly selective and limited substitution was unanticipated. As such, claims 1 and 20, and their related dependent claims, are not anticipated by D! and demonstrate a significant inventive step over D1.

The Applicant further notes that claims that included unacceptable multiple dependencies as submitted have been suitably amended.

For at least these reasons, the Applicant respectfully requests withdrawal of the rejections of claims 1 to 33.

Conclusion

Claims 1 to 33 are pending in this application. The Applicants request allowance of all pending claims.

SEQUENCE LISTING

<110> Nansha Biologics (Hong Kong)

<120> COMPOSITIONS AND METHODS FOR PEGYLATED IL-11

<130> 102635.0001PCT

<150> US 62/127,748
<151> 2015-03-03

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<223> human IL-11 lacking N-terminal P

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1 5 10 15

Leu Asp Ser Thr Val Leu Leu Thr Arg Ser Leu Leu Ala Asp Thr Arg
20 25 30

Gln Leu Ala Ala Gln Leu Arg Asp Lys Phe Pro Ala Asp Gly Asp His
35 40 45

Asn Leu Asp Ser Leu Pro Thr Leu Ala Met Ser Ala Gly Ala Leu Gly
50 55 60

Ala Leu Gln Leu Pro Gly Val Leu Thr Arg Leu Arg Ala Asp Leu Leu
65 70 75 80

Ser Tyr Leu Arg His Val Gln Trp Leu Arg Arg Ala Gly Gly Ser Ser
85 90 95

Leu Lys Thr Leu Glu Pro Glu Leu Gly Thr Leu Gln Ala Arg Leu Asp
100 105 110

Arg Leu Leu Arg Arg Leu Gln Leu Leu Met Ser Arg Leu Ala Leu Pro
115 120 125

Gln Pro Pro Pro Asp Pro Pro Ala Pro Pro Leu Ala Pro Pro Ser Ser
130 135 140

Ala Trp Gly Gly Ile Arg Ala Ala His Ala Ile Leu Gly Gly Leu His
145 150 155 160

Leu Thr Leu Asp Trp Ala Val Arg Gly Leu Leu Leu Leu Lys Thr Arg
165 170 175

Leu

10 20 30 40 50 60
GPPPGPPRVS PDPRAELDST VLLTRSLLAD TRQLAAQLRD KFPADGDHNL DSLPTLAMSA
Helix A

70 80 90 100 110 120
GALGALQLPG VLTRLRADLL SYLRHVQWLR RAGGSSLKTL EPELGTLQAR LDRLLRRLQL
Helix B Helix C

130 140 150 160 170
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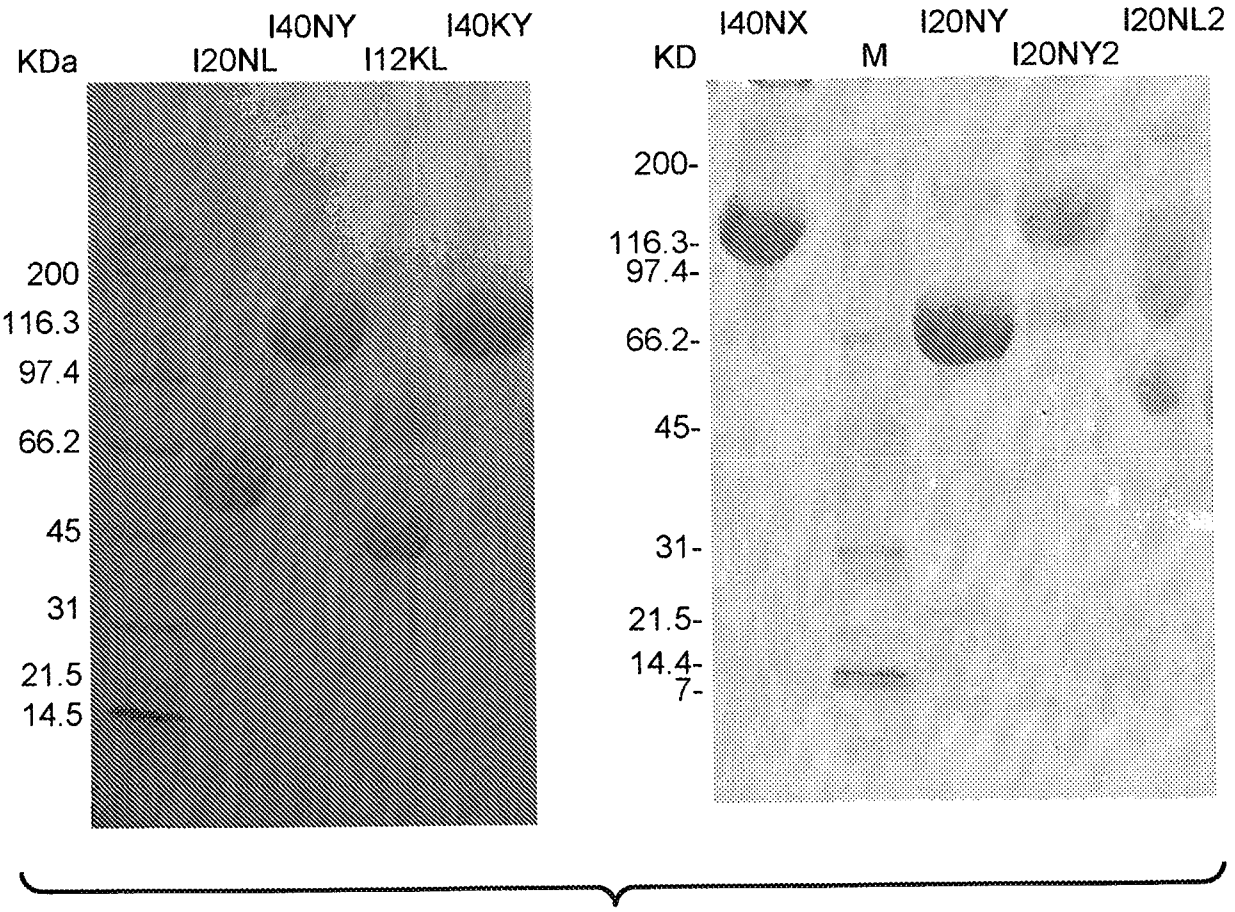
Helix D

FIG. 1

FIG. 2



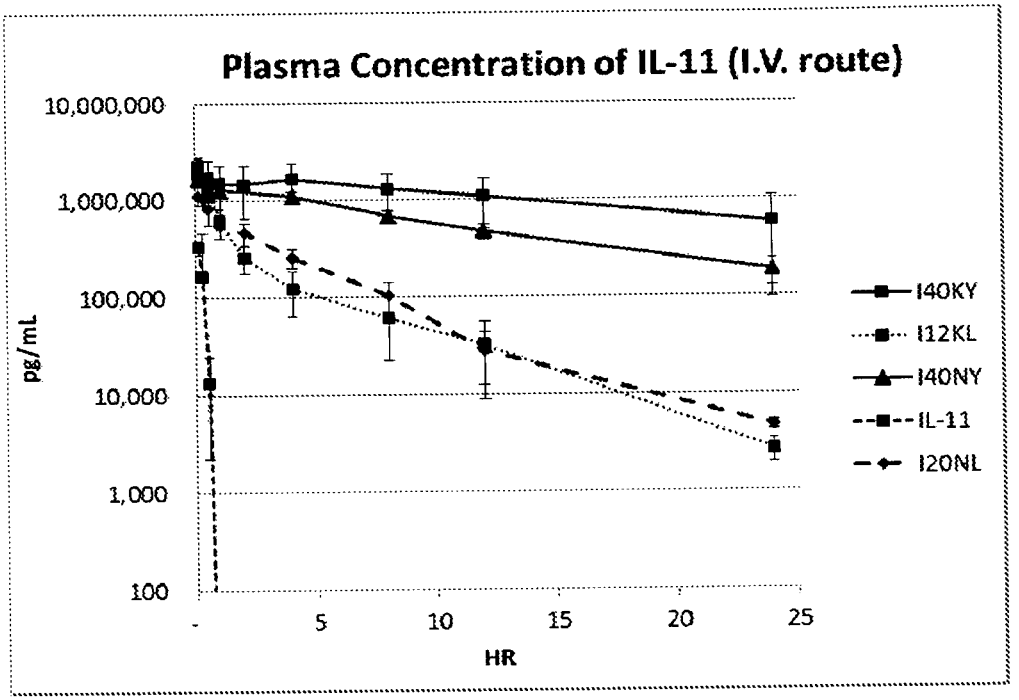


Figure 3

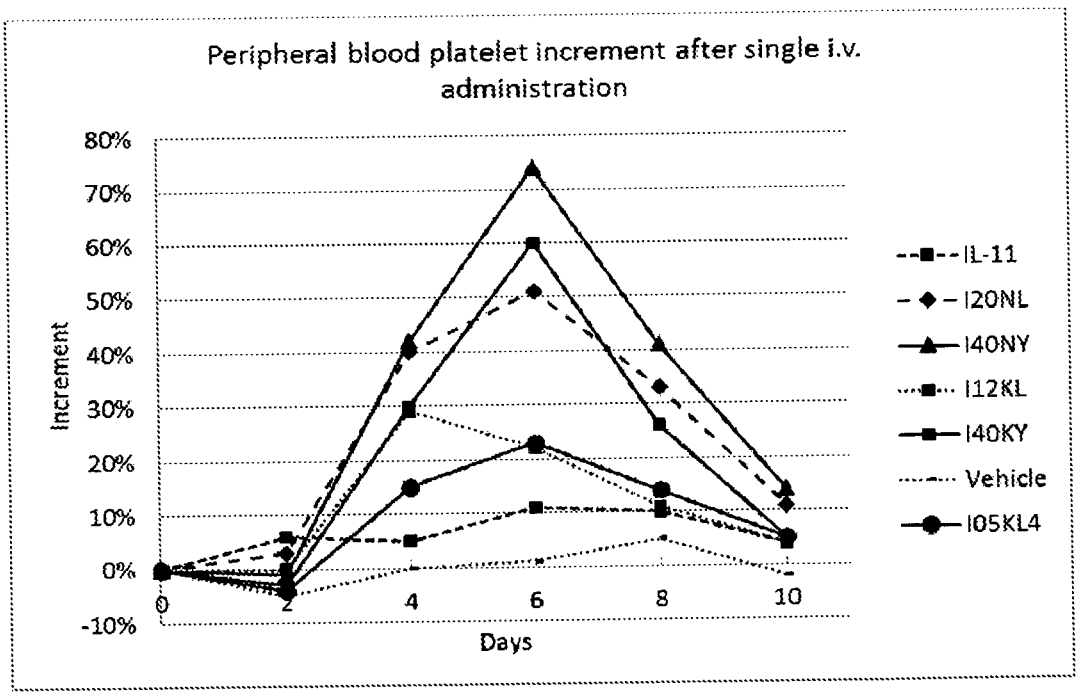
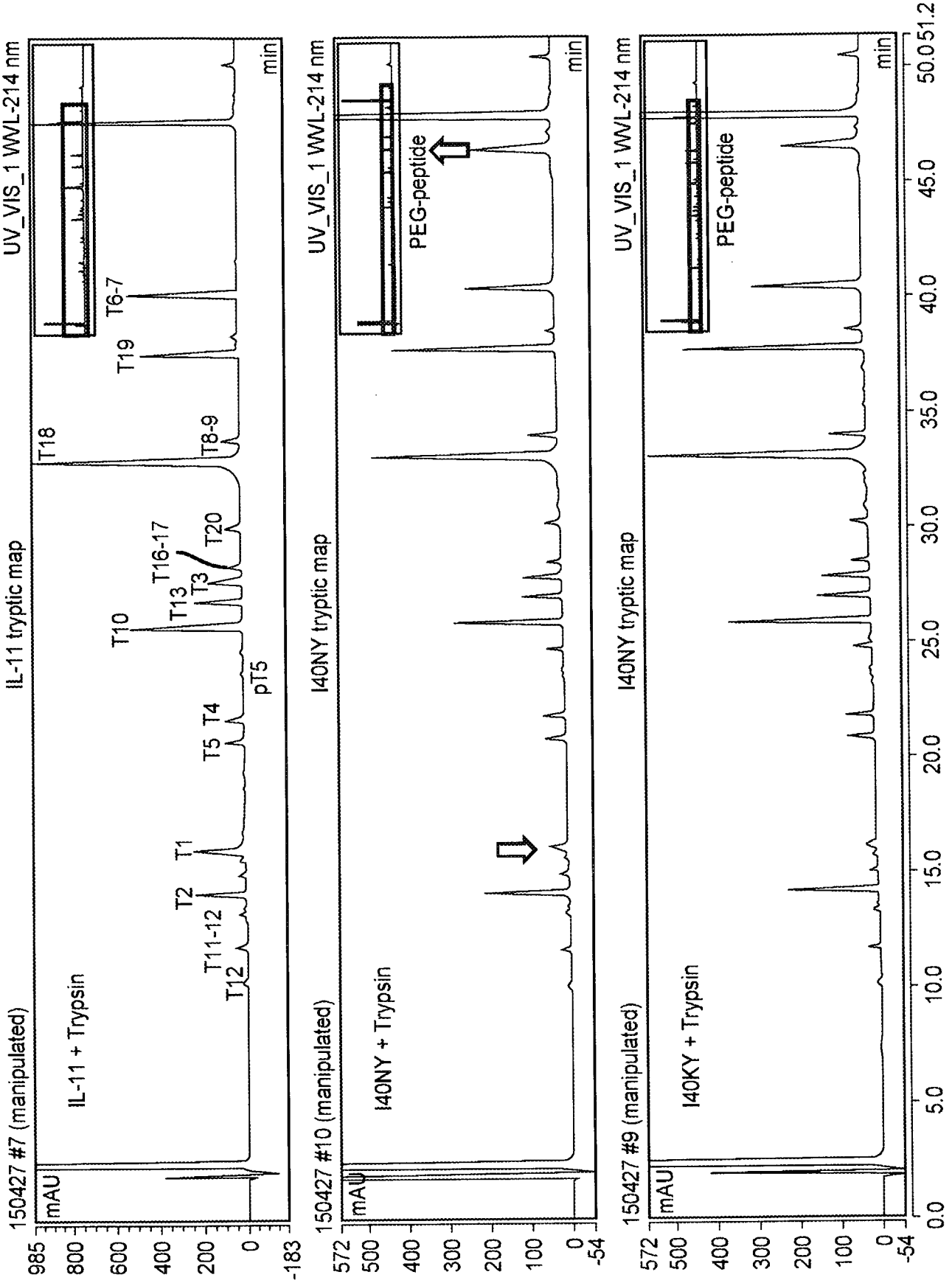


Figure 4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IL-11 + Trypsin
UV_VIS_1 WVL-214 nm
IL-11 tryptic map
mAU
985
800
600
400
200
0
-183
min
T18
T19
T6-7
T8-9
T10
T16-17
T13
T3
T20
T1
T2
T11-12
T12
T5
T4
pT5
150427 #10 (manipulated)
I40NY + Trypsin
UV_VIS_1 WVL-214 nm
I40NY tryptic map
PEG-peptide
572
500
400
300
200
100
0
-54
150427 #9 (manipulated)
I40KY + Trypsin
UV_VIS_1 WVL-214 nm
I40NY tryptic map
PEG-peptide
0.0
5.0
10.0
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20.0
25.0
30.0
35.0
40.0
45.0
50.0
51.2

FIG. 5

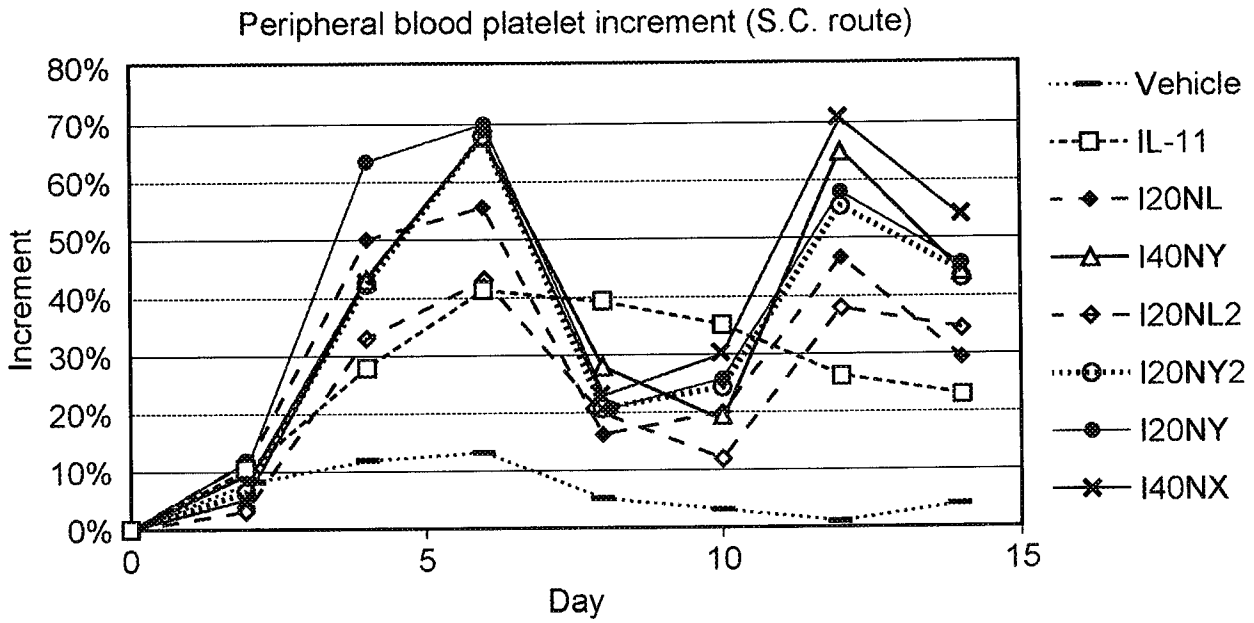

Peripheral blood platelet increment (S.C. route)
80%
70%
60%
50%
40%
30%
20%
10%
0%
Increment
0
5
10
15
Day
Vehicle
IL-11
I20NL
I40NY
I20NL2
I20NY2
I20NY
I40NX

FIG. 6

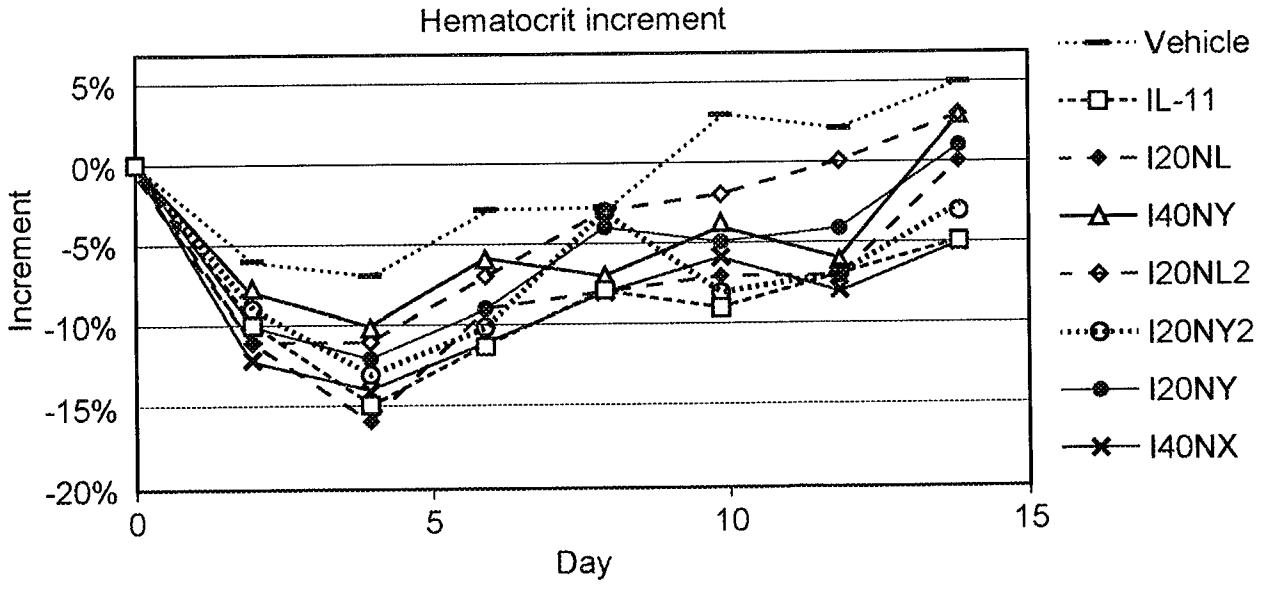

Hematocrit increment
5%
0%
-5%
-10%
-15%
-20%
Increment
0
5
10
15
Day
Vehicle
IL-11
I20NL
I40NY
I20NL2
I20NY2
I20NY
I40NX

FIG. 7

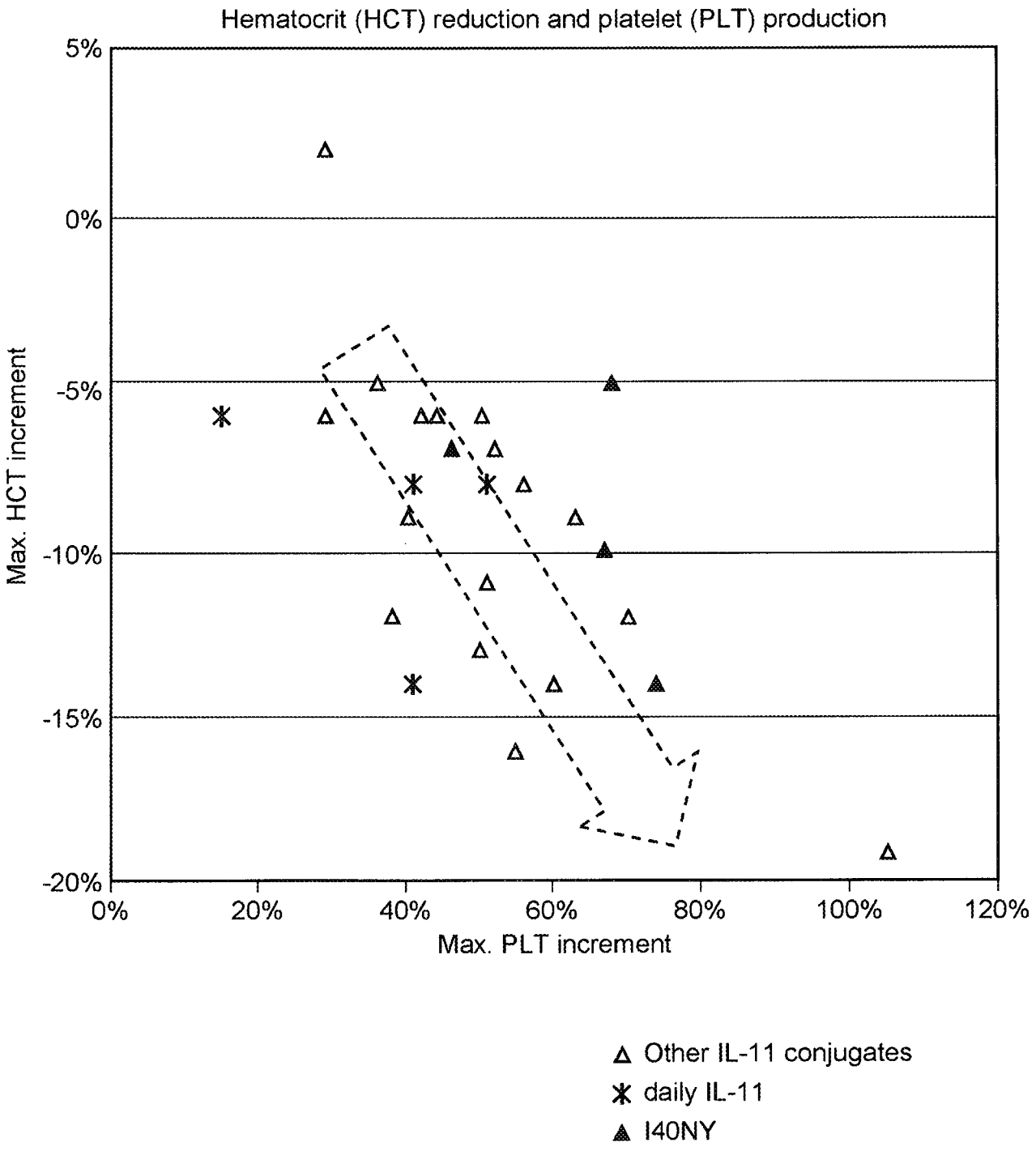
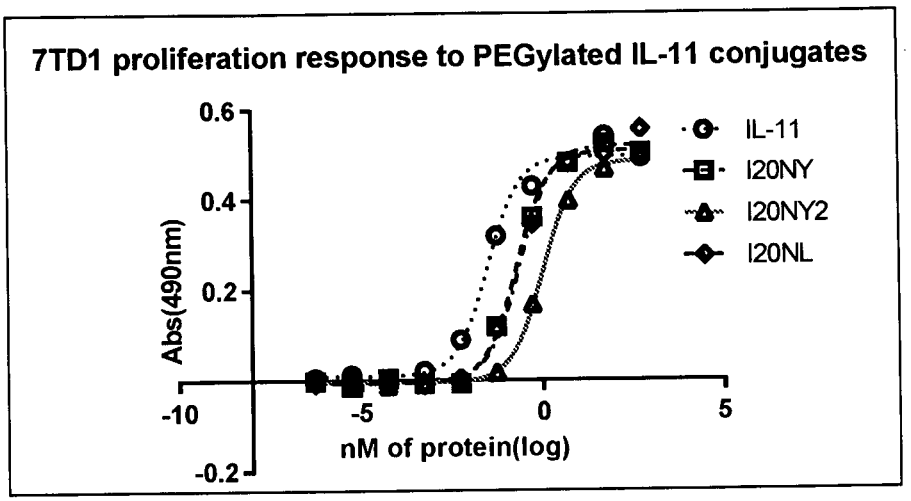
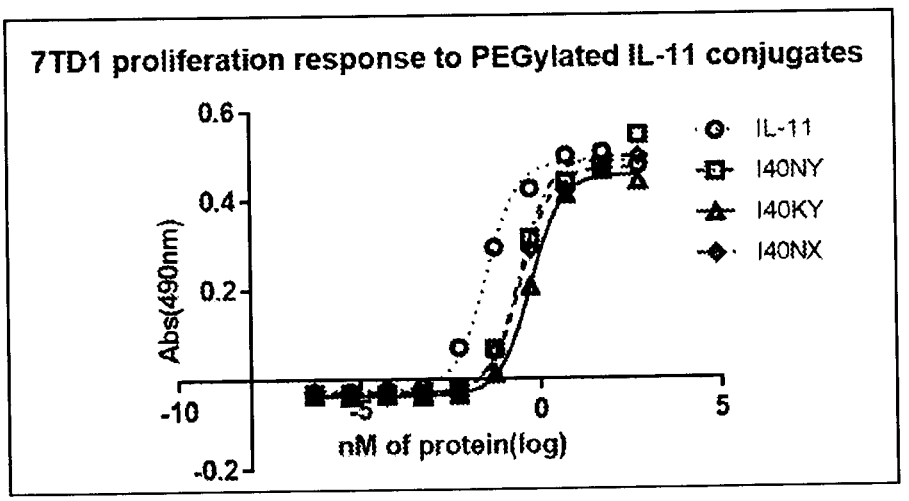

Hematocrit (HCT) reduction and platelet (PLT) production
5%
0%
-5%
-10%
-15%
-20%
Max. HCT increment
0%
20%
40%
60%
80%
100%
120%
Max. PLT increment
Other IL-11 conjugates
daily IL-11
I40NY

FIG. 8



	IL-11	I20NY	I20NY2	I20NL
EC50(pM)	30.49	191.60	1012.0	239.80
R square	0.9918	0.9979	0.9988	0.9954
Potency ratio to unmodified IL-11	1	0.16	0.03	0.13



	IL-11	I40NY	I40KY	I40NX
EC50(pM)	30.80	277.20	536.40	292.90
R square	0.9957	0.9926	0.9991	0.9968
Potency ratio to unmodified IL-11	1	0.11	0.06	0.11

Figure 9

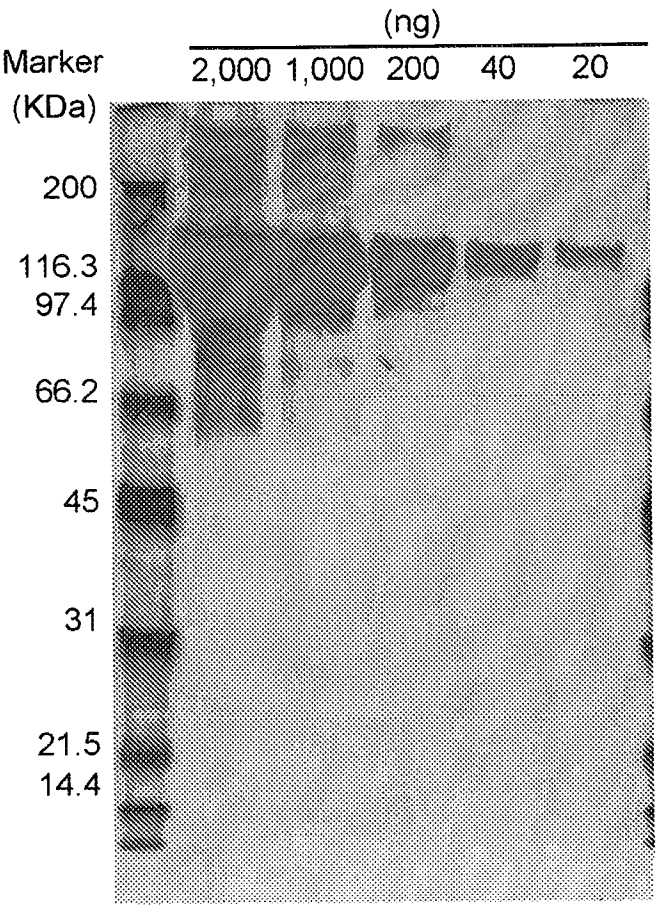

(ng)
Marker
(KDa)
2,000
1,000
200
40
20
200
116.3
97.4
66.2
45
31
21.5
14.4

FIG. 10

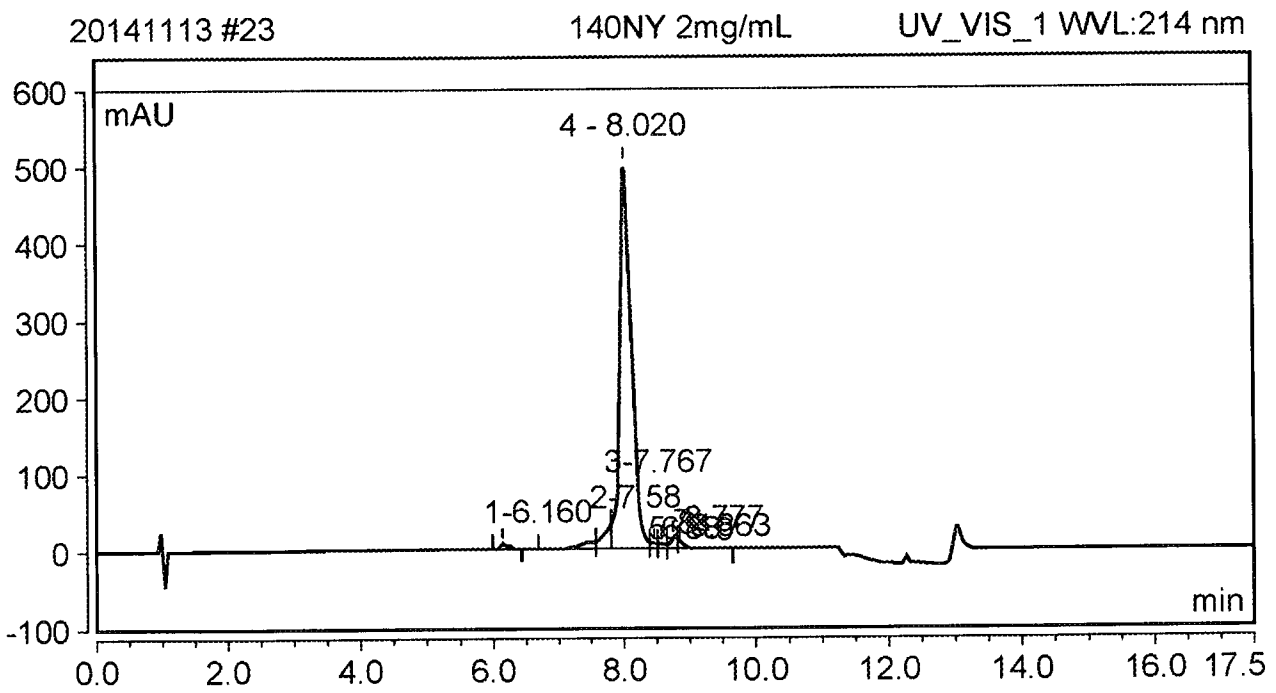

20141113 #23
140NY 2mg/mL
UV_VIS_1 WVL:214 nm
mAU
4 - 8.020
3-7.767
1-6.160
2-7.58
min
600
500
400
300
200
100
0
-100
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
17.5

FIG. 11

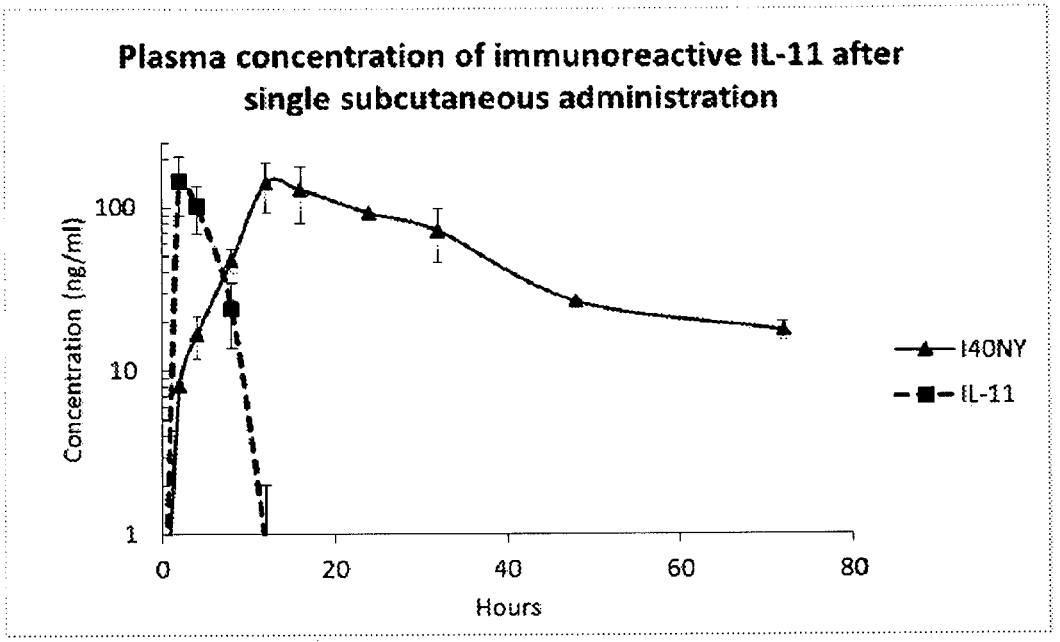


Figure 12

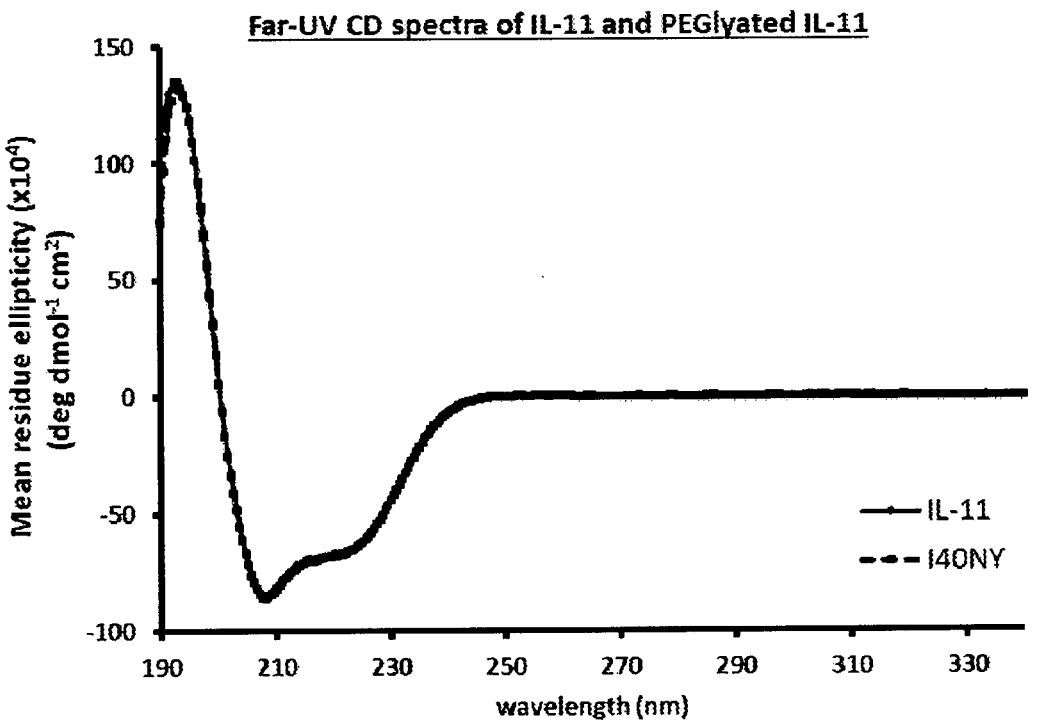


Figure 13

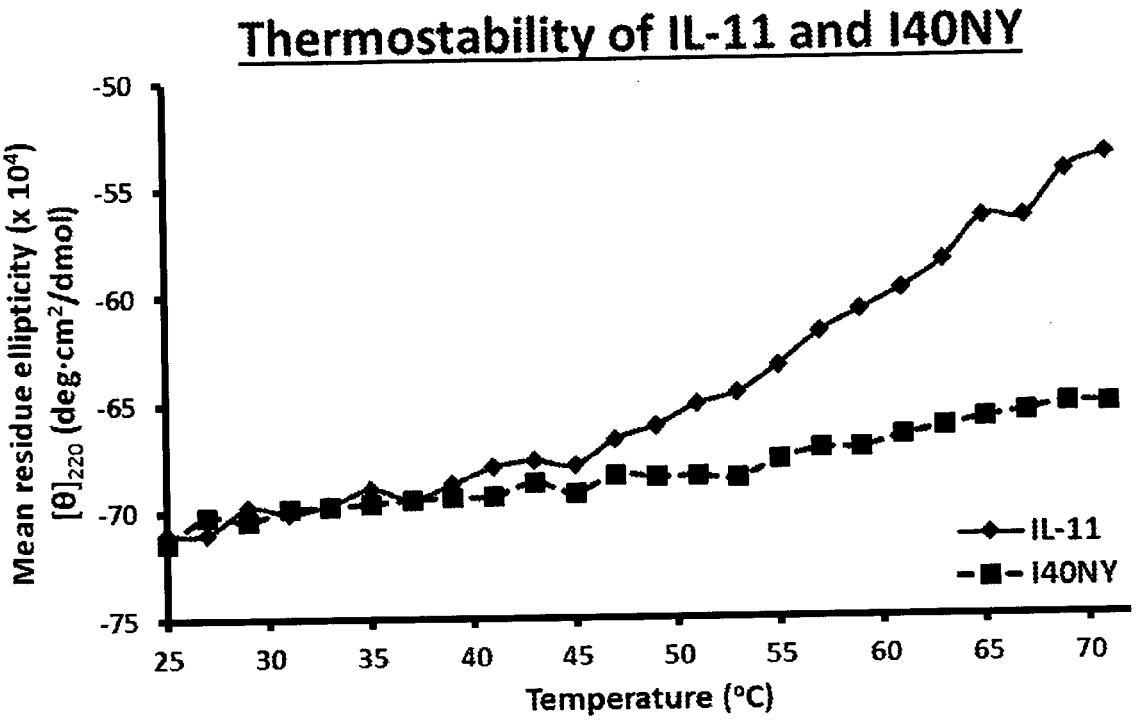

Thermostability of IL-11 and I40NY
Mean residue ellipticity (x 10⁴)
[θ]220 (deg·cm²/dmol)
-50
-55
-60
-65
-70
-75
25
30
35
40
45
50
55
60
65
70
Temperature (°C)
IL-11
I40NY

Figure 14

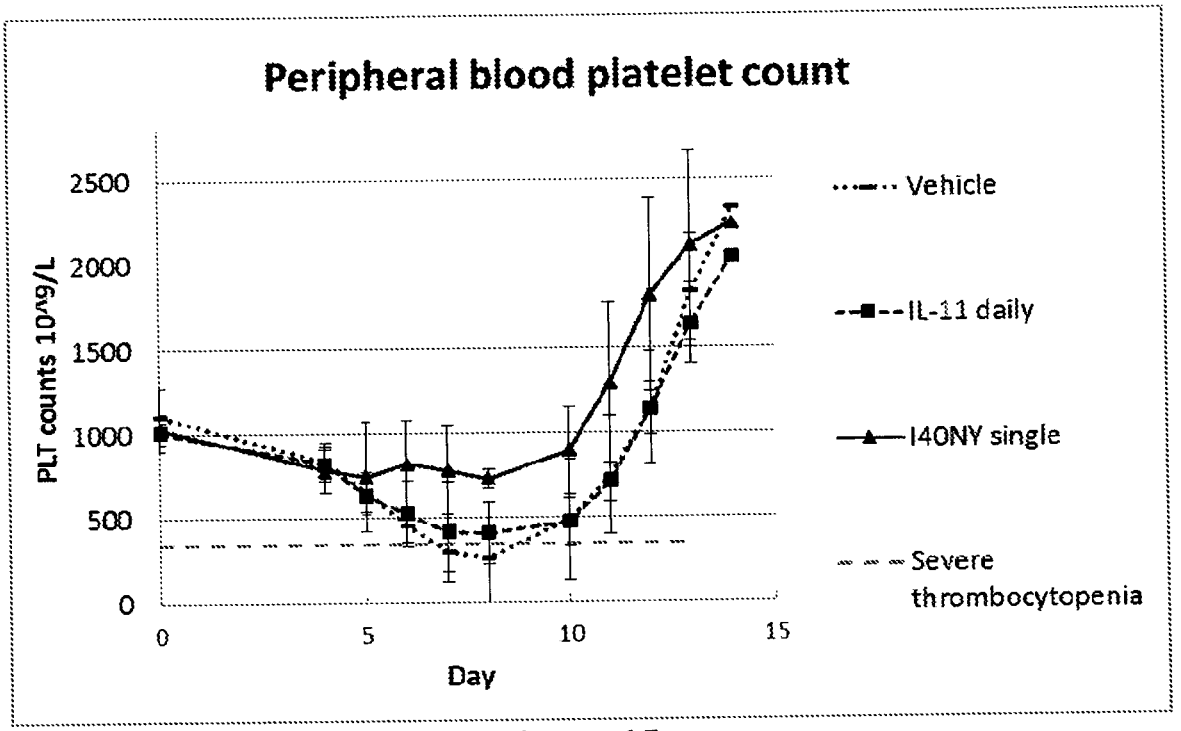


Figure 15

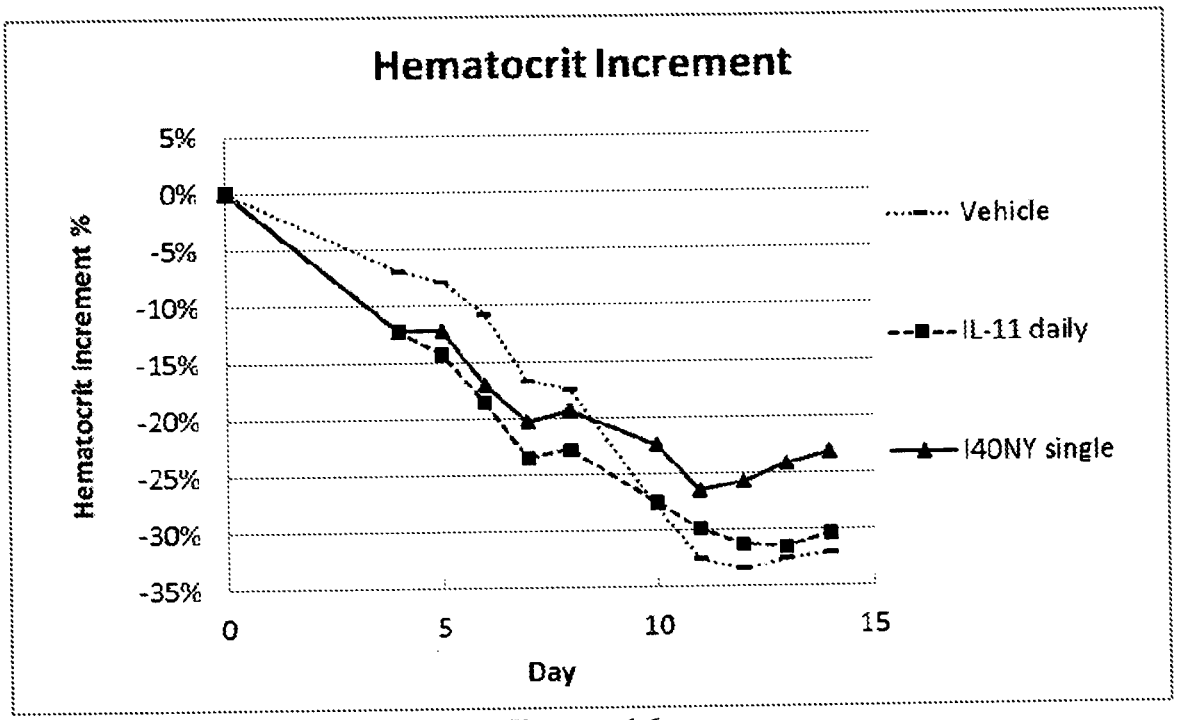


Figure 16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/020294

A. CLASSIFICATION OF SUBJECT MATTER

C07K 14/54(2006.01)i, A61K 38/20(2006.01)i, A61K 9/19(2006.01)i, A61K 47/28(2006.01)i, A61K 47/32(2006.01)i, A61P 1/00(2006.01)i, A61P 9/00(2006.01)i, A61P 11/00(2006.01)i

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)
C07K 14/54; A61K 38/00; A61K 45/00; A61K 38/20; A61K 9/19; A61K 47/28; A61K 47/32; A61P 1/00; A61P 9/00; A61P 11/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: modified human interleukin 11, N-terminal directed PEGylation, N-terminal proline deletion

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 2005-281302 A (ASTELLAS PHARMA INC) 13 October 2005 See claims 1-5; and paragraphs [0020]-[0031].	1-3,20-22
A	US 8716446 B2 (JUNG et al.) 06 May 2014 See abstract; claims 1-14; and column 12.	1-3,20-22
A	WO 2013-020079 A2 (NEKTAR THERAPEUTICS) 07 February 2013 See the whole document.	1-3,20-22
A	WO 2014-008242 A1 (THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK) 09 January 2014 See the whole document.	1-3,20-22
A	NCBI, PDB: 4MHL_A (22 October 2014) See the whole sequence.	1-3,20-22
L	Application No. PCT/US2016/020356 (AVALON BIOLOGICS LIMITED et al.) Application date: 02 March 2016	1-3,20-22

☐ 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	Date of mailing of the international search report
24 May 2016 (24.05.2016)	**01 June 2016 (01.06.2016)**

Name and mailing address of the ISA/KR
International Application Division
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon, 35208, Republic of Korea
Facsimile No. +82-42-481-8578

Authorized officer
KIM, Seung Beom
Telephone No. +82-42-481-3371

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/020294

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.: 29-33
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 29-33 pertain to methods for treatment of the human body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under PCT Article 17(2)(a)(i) and PCT Rule 39.1(iv), to search.

2. ☒ Claims Nos.: 11-15, 17-19, 29-30
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:
Claims 11-15, 17-19 and 29-30 refer to unsearchable claims which do not comply with PCT Rule 6.4(a).

3. ☒ Claims Nos.: 4-10, 16, 23-28, 31-33
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:

1. ☐ As all required addtional 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 an additional fees, this Authority did not invite payment of any 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.:

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.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2016/020294

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 2005-281302 A	13/10/2005	WO 2005-085283 A1	15/09/2005
US 8716446 B2	06/05/2014	AU 2009-284840 A1	04/03/2010
		AU 2009-284840 B2	27/09/2012
		CA 2730721 A1	04/03/2010
		CN 102131518 A	20/07/2011
		CN 102131518 B	20/01/2016
		EP 2334320 A2	22/06/2011
		EP 2334320 A4	28/03/2012
		EP 2334320 B1	10/02/2016
		JP 2012-500845 A	12/01/2012
		JP 5602738 B2	08/10/2014
		KR 10-1292740 B1	05/08/2013
		KR 10-2011-0051246 A	17/05/2011
		MX 2011-002055 A	30/03/2011
		US 2010-0098658 A1	22/04/2010
		WO 2010-024557 A2	04/03/2010
		WO 2010-024557 A3	24/06/2010
WO 2013-020079 A2	07/02/2013	WO 2013-020079 A3	18/04/2013
WO 2014-008242 A1	09/01/2014	None	

(19)中华人民共和国国家知识产权局

(12)发明专利申请

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2017.10.26

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WO2016/140983 EN 2016.09.09

(71)申请人 阿瓦隆生物制品有限公司
地址 中国香港

(72)发明人 K·余 Q·周 M·福克 J·Y·刘

(74)专利代理机构 北京纪凯知识产权代理有限公司 11245
代理人 张全信 董志勇

(51)Int.Cl.
C07K 14/54(2006.01)
A61K 38/20(2006.01)
A61K 47/28(2006.01)
A61K 47/32(2006.01)
A61P 9/00(2006.01)
A61P 11/00(2006.01)
A61P 1/00(2006.01)

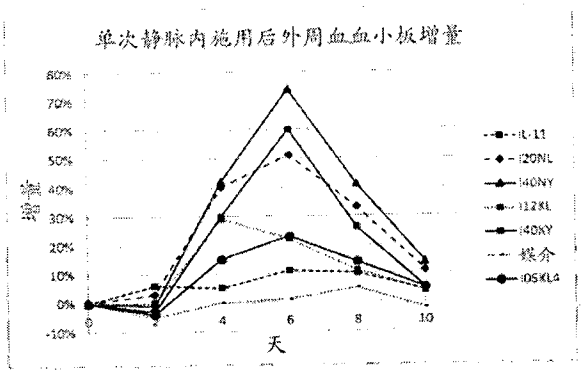
权利要求书2页 说明书20页 附图10页

(54)发明名称

聚乙二醇化的IL-11的组合物和方法

(57)摘要

提供了组合物和方法，其中重组IL-11被聚乙二醇化以取得改进的血清中的半衰期，同时具有期望的治疗活性并呈现更少的副作用。最优选地，IL-11是N端截短的人或人源化IL-11并且具有20Kd或40Kd支化的PEG部分，特别是Y形状的或梳子形状的，其偶联至N端氨基。这样的化合物的特征在于大幅度增加的血清中的稳定性和持久的生物学活性，同时展示显著降低的血浆膨胀。



CN 107849108 A

1.修饰的白介素11(IL-11)化合物,其包括:

共价偶联至PEG部分的IL-11多肽链,其中所述PEG部分具有10-50Kd之间的平均分子量并且具有不同的第一和第二PEG段;

其中所述PEG部分被共价结合至N端氨基酸;并且

其中所述IL-11多肽链是人或人源化多肽链。

2.权利要求1所述的修饰的化合物,其中所述IL-11多肽链是人IL-11多肽链。

3.权利要求1或2中任一项所述的修饰的化合物,其中所述IL-11多肽链通过缺失N端脯氨酸被缩短。

4.在先权利要求中任一项所述的修饰的化合物,其中所述IL-11多肽链具有根据SEQ ID NO:1的序列。

5.在先权利要求中任一项所述的修饰的化合物,其中所述PEG部分具有20Kd或40Kd的平均分子量。

6.在先权利要求中任一项所述的修饰的化合物,其中所述PEG部分具有Y形状。

7.在先权利要求中任一项所述的修饰的化合物,其中多肽链与PEG部分的摩尔比是大约1:1。

8.在先权利要求中任一项所述的修饰的化合物,进一步包括经由所述IL-11多肽链的氨基共价偶联的第二PEG部分。

9.在先权利要求中任一项所述的修饰的化合物,其中所述PEG部分经由胺键被共价结合至所述N端氨基酸。

10.药物组合物,其包括治疗有效量的根据权利要求1-9中任一项所述的IL-11化合物联合药学上可接受的载体。

11.权利要求10所述的药物组合物,其中所述组合物配制用于注射。

12.权利要求10所述的药物组合物,其中所述IL-11化合物以向儿童或成人患者提供10-100μg/kg之间的剂量单位的量存在。

13.权利要求10所述的药物组合物,其中所述组合物是冻干的。

14.权利要求10所述的药物组合物,进一步包括第二药学活性化合物。

15.权利要求14所述的药物组合物,其中所述第二药学活性化合物是类固醇、刺激骨髓中血小板产生的药剂、抗体、止痛剂或抗炎剂。

16.根据权利要求1-9中任一项所述的IL-11化合物在制造药物组合物中的用途。

17.权利要求16所述的用途,其中所述药物组合物用于治疗选自如下的病症:(a)核事故/辐射诱发的骨和胃肠道损害;(b)化疗诱发的骨和胃肠道损害;(c)烧伤诱发的血小板减少和胃肠道损害;(d)化疗诱发的血小板减少;(e)创伤、癌症或感染诱发的胃肠道损害或炎性肠道疾病;(f)自由基诱发的肺损害;和(g)心血管疾病。

18.权利要求16所述的用途,其中所述药物组合物配制用于注射。

19.权利要求16所述的用途,其中所述药物组合物是冻干的。

20.增加白介素11(IL-11)化合物的血清半衰期的方法,其包括:

将IL-11多肽链共价偶联至PEG部分,其中所述PEG部分具有10-50Kd之间的平均分子量并且具有不同的第一和第二PEG段;

其中所述PEG部分被共价结合至N端氨基酸;并且

其中所述IL-11多肽链是人或人源化多肽链。

21.权利要求20所述的方法，其中所述IL-11多肽链是人IL-11多肽链。

22.权利要求20-21中任一项所述的方法，其中所述IL-11多肽链通过缺失N端脯氨酸被缩短。

23.权利要求20-22中任一项所述的方法，其中所述IL-11多肽链具有根据SEQ ID NO:1的序列。

24.权利要求20-23中任一项所述的方法，其中所述PEG部分具有20Kd或40Kd的平均分子量。

25.权利要求20-24中任一项所述的方法，其中所述PEG部分具有Y形状。

26.权利要求20-25中任一项所述的方法，其中多肽链与PEG部分的摩尔比是大约1:1。

27.权利要求20-26中任一项所述的方法，进一步包括经由所述IL-11多肽链中的氨基共价偶联第二PEG部分的步骤。

28.权利要求20-27中任一项所述的方法，其中所述PEG部分经由胺键被共价结合至所述N端氨基酸。

29.治疗响应于施用IL-11的病症的方法，其包括以治疗有效量给有需要的患者施用权利要求10所述的药物组合物的步骤。

30.权利要求29所述的方法，其中所述病症选自(a)核事故/辐射诱发的骨和胃肠道损害；(b)化疗诱发的骨和胃肠道损害；(c)烧伤诱发的血小板减少和胃肠道损害；(d)化疗诱发的血小板减少；(e)创伤、癌症或感染诱发的胃肠道损害或炎性肠道疾病；(f)自由基诱发的肺损害；和(g)心血管疾病。

31.权利要求29或30所述的方法，其中所述药物组合物包括IL-11I40NY或I20NY。

32.权利要求29-31中任一项所述的方法，其中所述IL-11以10-100μg/kg之间的剂量施用。

33.权利要求29-32中任一项所述的方法，其中所述IL-11被皮下施用。

聚乙二醇化的IL-11的组合物和方法

[0001] 本申请要求2015年3月3日提交的序列号为62/127748的本申请人共同未决的美国临时申请的优先权，并且其通过引用并入本文。

技术领域

[0002] 本发明的领域是药物组合物和方法，尤其是当它们涉及聚乙二醇化的(PEGylated)白介素11(IL-11)时。

背景技术

[0003] 背景描述包括可能有助于理解本发明的信息。这并非承认在本文提供的任何信息是现有技术或与本发明相关，也并非承认明确地或隐含地引用的任何出版物是现有技术。本文中的所有出版物通过引用并入，如同每个单独的出版物或专利申请被明确地且单独地指示通过引用并入一样。在并入的参考文献中的术语的定义或使用与本文提供的该术语的定义不一致或相反时，本文提供的该术语的定义适用，而参考文献中的该术语的定义不适用。

[0004] 化疗诱发的血小板减少仍然是未满足的医疗需求，这是因为目前的治疗方案采用血小板的输注，其可能供应短缺并且带有病毒污染的风险。另一方面，可以给予患者重组人IL-11以刺激血小板产生。然而，IL-11施用需要每日给药，其导致边际临床疗效(marginal clinical efficacy)和血浆膨胀。

[0005] IL-11是细胞因子，并且在血细胞生成中且尤其在巨核细胞成熟的刺激中充当主要的信号传导剂。IL-11的作用通常由IL-11受体和糖蛋白gp130——利用gp130后续的磷酸化/活化——介导。IL-11的临床用途包括治疗与化疗相关联的副作用，其被认为增强巨核细胞生成和增加血小板计数。重组人IL-11作为NEUMEGA®(奥普瑞白介素，Wyeth-Ayerst)是商业上可获得的，并且被批准用于预防严重的血小板减少和降低患有非骨髓性恶性肿瘤的成年患者——其处于严重的血小板减少的高风险下——在骨髓抑制性化疗之后对血小板输注的需求。NEUMEGA®通常以单次使用的小瓶供应，其包含5mg作为冻干粉末的IL-11，使用1mL无菌水重构用于注射(以25-50μg/kg/天的剂量施用)。最常见的与NEUMEGA™相关联的不良事件是血浆膨胀，其导致威胁生命的房性心律失常、晕厥、呼吸困难、充血性心力衰竭和肺水肿。

[0006] IL-11相对快速地从循环统被清除，并且因此需要多次注射。例如，在健康男性中皮下施用的Neumega™具有大约6.9小时的终末半衰期(terminal half-life)(Neumega™的产品说明书)。差的药代动力学比如快速的肾排泄和蛋白水解消化，以及其相关联的不利效应通常降低临床普及。而且，每日注射也意味着住院治疗以管理不良事件，其不仅增加医疗费用而且损害患者的生活质量。结果，血小板输注仍然是用于治疗化疗诱发的血小板减少(CIT)的黄金标准。

[0007] 为了增加血清稳定性同时维持这样的组合物的有益的治疗潜力，本领域已经进行了多种尝试。例如，US 2010/0098658报道了与聚合物(PEG)缔合的IL-11类似物(mIL-11)，

其展示出增强的对酸解的抗性和增加的血清半衰期。在稳定IL-11的另一个尝试中，如US8133480中描述的，制备了IL-11的半胱氨酸变体并且使用PEG进一步修饰选择的突变蛋白质以增加血清稳定性。虽然这些修饰已经在至少一些程度上改进了IL-11的血清稳定性或半衰期，但是仍存在一个或多个劣势，其包括骨髓抑制性动物中的边际功效、生产的复杂性、重复给药、和配制为可注射的溶液。

[0008] 由于IL-11中缺少半胱氨酸残基，'480专利描述了在C端氨基酸序列中插入半胱氨酸残基，其给予官能团以使得硫醇-反应性聚乙二醇链缀合。虽然生物学活性得以保存，但是引入半胱氨酸可以产生分子间二聚体，并且昆虫细胞的生产产量可能低于细菌产生。此外，当在雄性SD大鼠中静脉内施用时，对于40KD聚乙二醇化，如此修饰的IL-11的血清半衰期是大约5.6hr，这比期望的小。而且，在使用环磷酰胺处理的大鼠的动物研究中，使用几天前(the-other-day)给药方案的功效是边际的。在US 2010/0098658中描述了另一种采用将20KD PEG经由胺或酰胺键合PEG缀合至IL-11的N端截短序列上。虽然N端截短并不降低其生物学活性，但是在雄性SD大鼠中皮下施用的血清半衰期是大约8.5hr，也未达到期望的稳定性。此外，在动物疾病模型中的功效未知。

[0009] 报道了缀合至IL-11的胺基团上的20KD的直链或支化PEG(Takagi et al.2007, "Enhanced pharmacological activity of recombinant human interleukin-11 (rhIL11)by chemical modification with polyethylene glycol."J Control Release, 119(3):271-278)，这样的非特异性缀合通常经由与赖氨酸、组氨酸和酪氨酸残基以及N端胺的反应导致多重聚乙二醇化。

[0010] 其它报道已经展现出IL-11的"非核心"区比如N端和环上的某些碳水化合物修饰增强细胞刺激活性，这表明可能设计这些区来限制IL-11的生物学活性(Yanaka et al.2011,"Non-core region modulates interleukin-11signaling activity: generation of agonist and antagonist variants."J.Biol.Chem.,286:8085-8093)。然而，没有报道具有优于未修饰的IL-11的稳定性和活性的期望的修饰。

[0011] 因而，即使在本领域中已知几种稳定IL-11的方法，但是所有或几乎所有均具有一个或多个缺点，比如有限的功效和需要重复给药。更重要地，甚至在修饰的形式中，也没有降低IL-11的不利效应(例如，血浆膨胀)。因此，对稳定IL-11同时减轻不利效应的改进的组合物和方法仍然存在需要。

发明内容

[0012] 本发明主题涉及用于改进IL-11在血清中的稳定性和半衰期同时维持生物学活性和减轻副作用的化合物、组合物和方法。在尤其优选的方面，本发明人已经发现氨基酸位置、连接的方式、和PEG的类型对产生稳定的和生物活性的聚乙二醇化的IL-11是至关重要的，并且特别优选的聚乙二醇化的IL-11将具有与天然人IL-11相同但是缺少N端第一个氨基酸脯氨酸的序列。而且，这样的IL-11将优选地在N端处以及多肽链内某些赖氨酸残基处可能的二级位点(secondary site)处共价修饰。最典型地，IL-11与连接至IL-11的PEG化合物的平均摩尔比是1:1。

[0013] 在本发明主题的一方面，本发明人考虑了修饰的白介素11(IL-11)化合物，其包括共价偶联至PEG部分的IL-11多肽链，其中PEG部分具有10-50Kd之间的平均分子量并且具有

不同的第一和第二PEG段，其中PEG部分被共价结合至N端氨基酸，并且其中IL-11多肽链是人或人源化多肽链。

[0014] 最典型地，IL-11多肽链是人IL-11多肽链，和/或可以通过缺失N端脯氨酸被缩短。例如，尤其合适的IL-11多肽链可以具有根据SEQ ID NO:1的序列。关于PEG部分，通常优选的是该部分具有20Kd或40Kd的平均分子量，和/或该PEG部分具有Y形状。虽然不限制本发明主题，但是优选的是多肽链与PEG部分的摩尔比是大约1:1(例如:0.9:1至1:0.9，或0.8:1至1:0.8)。此外，考虑第二PEG部分可以经由IL-11多肽链的氨基被共价偶联至修饰的IL-11。另外，通常优选的是PEG部分经由胺键被共价结合至N端氨基酸(然而，也明确地考虑酰胺键)。

[0015] 从另一个角度来看，本发明人还考虑药物组合物，其包括治疗有效量的根据本发明主题的IL-11化合物(例如，如上面描述的)联合药学上可接受的载体。期望的是组合物可以配制用于注射，并且可以包括以向儿童或成人患者提供10-100μg/kg之间的剂量单位的量存在的IL-11化合物。此外，考虑组合物可以是冻干的或处于液体形式用于注射或输注。最合适的是药物组合物可以进一步包括第二药学活性化合物，其与IL-11化合物分离或混合。因而，本文还明确地考虑包括考虑的药物组合物连同其它组分(例如，第二药学活性化合物比如类固醇、刺激骨髓中血小板产生的药剂、抗体、止痛剂、抗炎剂或用于重构的溶剂)的试剂盒。

[0016] 因此，本发明人还考虑根据本发明主题的IL-11化合物在制造药物组合物中的用途。虽然不限制本发明主题，但是尤其考虑的治疗包括(a)核事故/辐射诱发的骨和胃肠道损害；(b)化疗诱发的骨和胃肠道损害；(c)烧伤诱发的血小板减少和胃肠道损害；(d)化疗诱发的血小板减少；(e)创伤、癌症或感染诱发的胃肠道损害或炎性肠道疾病；(f)自由基诱发的肺损害；和(g)心血管疾病。如前面记载的，通常考虑药物组合物配制用于注射和/或药物组合物是冻干的。

[0017] 在本发明主题的进一步的方面，本发明人还考虑增加白介素11(IL-11)化合物的血清半衰期的方法。优选的方法将包括将IL-11多肽链共价偶联至PEG部分的步骤，其中PEG部分具有10-50Kd之间的平均分子量并且具有不同的第一和第二PEG段，其中PEG部分被共价结合至N端氨基酸，并且其中IL-11多肽链是人或人源化多肽链。最典型地，IL-11多肽链是人IL-11多肽链，和/或IL-11多肽链通过缺失N端脯氨酸被缩短(例如，具有根据SEQ ID NO:1的序列)。

[0018] 在进一步考虑的方法中，PEG部分具有20Kd或40Kd的平均分子量，和/或可以具有Y形状。期望的是多肽链与PEG部分的摩尔比是大约1:1，并且进一步考虑该方法可以进一步包括经由IL-11多肽链中的氨基共价偶联第二PEG部分的步骤。如前面，考虑PEG部分经由胺键被共价结合至N端氨基酸。

[0019] 在进一步考虑的方法中，本发明人考虑治疗响应于施用IL-11的病症的方法。这样的方法将通常包括以治疗有效量给有需要的患者施用考虑的药物组合物的步骤。例如，合适的病症可选自(a)核事故/辐射诱发的骨和胃肠道损害；(b)化疗诱发的骨和胃肠道损害；(c)烧伤诱发的血小板减少和胃肠道损害；(d)化疗诱发的血小板减少；(e)创伤、癌症或感染诱发的胃肠道损害或炎性肠道疾病；(f)自由基诱发的肺损害；和(g)心血管疾病。用于这些方法的示例性优选药物组合物可以包括IL-11I40NY或I20NY，并且进一步考虑IL-11以

10-100μg/kg之间的剂量施用(例如,皮下地)。

[0020] 根据下列优选实施方式的详细说明连同附图,本发明主题的多种目标、特征、方面和优点将变得更显而易见,在附图中相同的数字代表相同的组件。

附图说明

[0021] 图1描绘了不具有N端脯氨酸的IL-11的一级序列。

[0022] 图2是SDS-PAGE凝胶的图像,其具有分子量标记和如指示的多种聚乙二醇化形式的IL-11。

[0023] 图3是描绘在单次静脉内施用后多种IL-11组合物的血浆浓度的图表。

[0024] 图4是描绘在单次静脉内施用多种IL-11组合物后血小板增量的图表。

[0025] 图5以比较方式描绘了未缀合的IL-11、I40NY和I40KY的胰蛋白酶消化的肽图的层析图。

[0026] 图6是描绘在皮下施用多种IL-11组合物后血小板增量的图表(连续14天每天注射IL-11;和每周注射聚乙二醇化的对应物(counterpart))。

[0027] 图7是描绘在皮下施用多种IL-11组合物后血细胞比容降低的图表(连续14天每天注射IL-11;和每周注射聚乙二醇化的对应物)。

[0028] 图8是表明最大血小板诱发和最大血细胞比容降低之间的相关性的图表。

[0029] 图9是描绘在7TD1试验中聚乙二醇化的化合物的细胞增殖活性的图表,其与未缀合的IL-11进行比较。

[0030] 图10是使用银染色的非还原性SDS-PAGE凝胶的图像,其图解了在多种装载量下I40NY的纯度。

[0031] 图11是HPLC层析图,其描绘了I40NY的单-聚乙二醇化的组分的产品纯度。

[0032] 图12是药代动力学概况,其描绘了在单次皮下施用后I40NY的血浆浓度的动力学,其与单次皮下施用未缀合的IL-11进行比较。

[0033] 图13是IL-11和I40NY的圆二色谱的叠加。

[0034] 图14是作为温度的函数的IL-11和I40NY的椭圆率图。

[0035] 图15是药效动力学概况,其描绘了在骨髓抑制性大鼠的动物模型中考虑的化合物的血小板产生。

[0036] 图16是描绘在骨髓抑制性大鼠的动物模型中考虑的化合物的血细胞比容降低的图表。

具体实施方式

[0037] 本发明人已经发现PEG化合物的类型、共价连接的位置、和IL-11的一级序列是如此修饰的IL-11的稳定性和活性的决定因素。在特别优选和出乎意料的方面,本发明人发现当在N端截短一个氨基酸——其然后被聚乙二醇化——时,IL-11具有大幅改进的稳定性。此外,如下面更详细地进一步描述的,本发明人还发现PEG部分的具体类型和分子量是稳定性、活性和毒性的额外的决定因素。

[0038] 考虑的化合物

[0039] 为了研究PEG的类型、分子量和连接位置对于IL-11的影响,本发明人由重组人IL-

11——其具有如图1中显示的一级序列(与天然人IL-11序列相同,但是缺少N端脯氨酸)——制备了多种聚乙二醇化的IL-11分子。通常优选的是IL-11蛋白是N端截短的或修饰的人IL-11。例如,尤其优选的截短形式包括缺少至少一个或二个或三个(或更多个)N端氨基酸的IL-11分子。可选地,IL-11还可以被修饰以具有与人未修饰的对应物不同的N端氨基酸。例如,修饰的IL-11可以缺少第一个N端氨基酸并且可以具有第二个氨基酸,其不同于在未修饰的人IL-11中发现的第二个氨基酸(例如,缺少P并且使V替换G)。最典型地,N端氨基酸将是稳定的氨基酸并且因此尤其包括M、G、A、S、T、V或P,并且在进一步考虑的方面,不稳定的氨基酸(例如,F、Q、N、R等)可以由稳定的氨基酸替换。从N末端缺失一个或多个氨基酸将通常被限制于前十个、或前五个、或前三个氨基酸。另一方面,在较不优选的方面,也可以在IL-11部分的C端处实施一个、两个、三个、四个、五个或更多个氨基酸的缺失。作为一般性指导,缺失将通常被限于如下那些:其不影响或仅温和地不利影响生物学活性和/或稳定性(例如,失去小于20%和更典型地小于10%的活性和/或稳定性)。可选地或额外地,考虑的IL-11分子还包括与IL-11的融合蛋白,示例性融合蛋白包括US2010/0143973中描述的那些,其通过引用并入本文。最典型地,IL-11是重组蛋白并且可以在合适的表达系统中和最优选地在原核系统(例如,大肠杆菌)或酵母系统(例如,巴斯德毕赤酵母(Pichia pasteuris))中表达。当然,也应当认识到特别优选的IL-11的形式是成熟形式(即,不具有前导序列)。

[0040] 而且,应当领会合适的IL-11分子不必然是人IL-11,而是可以具有任何其它(通常是哺乳动物)起源。因此,合适的IL-11来源(重组的或天然的)包括灵长类、鼠、猪、马等。然后,这些序列可以被至少部分地人源化以降低免疫原性和/或增加在人中的稳定性和/或活性。类似地,本文还考虑合成的共有序列。

[0041] 考虑的IL-11分子的聚乙二醇化可以以众多方式进行并且包括共价以及非共价方法。然而,通常优选的是聚乙二醇化使用共价结合至IL-11。存在本领域已知的众多方式以将PEG基团共价连接至蛋白质,并且合适的方法包括如下那些:使N端氨基或C端羧酸基团与PEG部分上合适的反应基团(例如,醛、马来酰亚胺、酰基氯等)反应,或与巯基反应基团(例如,马来酰亚胺、吡啶基二硫化物(pyridyl disulfide)、乙烯基砜等)反应——其使二硫化物键合至半胱氨酸基团,或与氨基反应试剂反应——其与赖氨酸氨基酸的ε-氨基反应(例如,NHS-酯、NHS-碳酸酯、三嗪基团等)。因此,还考虑一个或多个氨基酸可以被添加至N端和/或C端以引入适于连接聚乙二醇化基团的反应基团。例如,可以添加丝氨酸或苏氨酸以允许使用N-乙酰半乳糖胺或PEG唾液酸衍生物的酶促连接,或添加赖氨酸用于共价连接至ε-氨基,或添加苯基丙氨酸或苏氨酸基团用于连接至羟基。

[0042] 关于本文使用的合适的PEG分子,通常考虑PEG的多种分子量是适当的,并且考虑的分子量在2Kd和200Kd之间(平均或标称分子量)。然而,特别优选的分子量(平均或标称分子量)包括在聚乙二醇化部分中每个直链在10-50Kd之间的那些。而且,通常优选的是PEG部分将具有单个直链的或Y形状的PEG部分,并且甚至更优选地,这样的PEG部分将具有20-40Kd之间的分子量。可选地,合适的PEG部分还可以包括树枝状PEG构建体,以及具有多于两个直链的PEG部分。在PEG部分具有多于一个直链PEG链时,通常优选的是链具有基本上相同的平均分子量(平均分子量差异小于15%)。

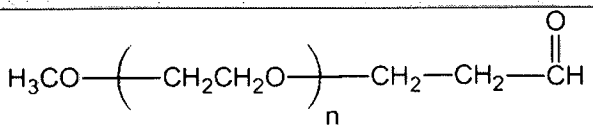
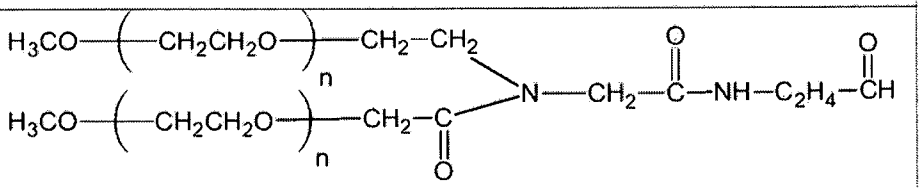
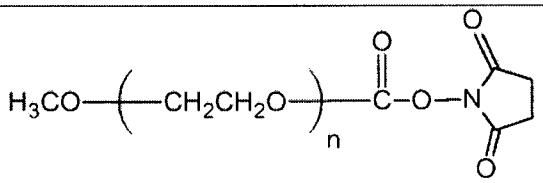
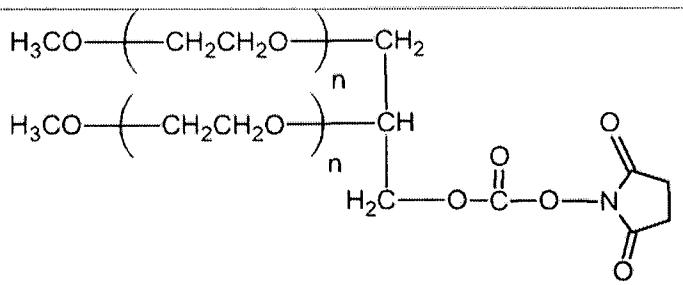
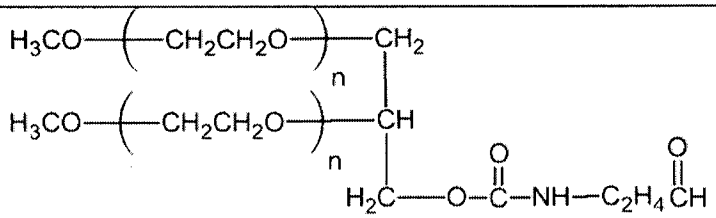
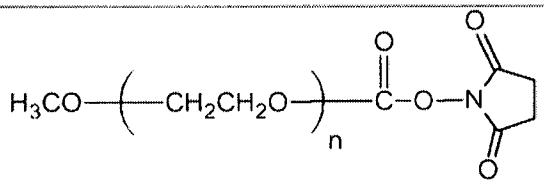
[0043] 在进一步优选的方面,PEG部分经由IL-11的N端氨基共价连接至IL-11和/或(任选

地)共价连接至内部赖氨酸的ε-氨基或组氨酸的环氮。由于N端共价键,优选的是IL-11与PEG部分的摩尔比是大约1:1(例如,0.9:1至1:0.9,或0.8:1至1:0.8等)。此外,应当领会中等水平的聚乙二醇化可以在内部氨基酸残基处存在(例如,10%-20%之间或1%-10%之间的所IL-11可以携带额外的聚乙二醇化的内部氨基酸)。例如,第二PEG部分可以被连接至内部赖氨酸的ε-氨基或组氨酸。如下面更详细地进一步显示的,特别优选的聚乙二醇化的IL-11的形式是I40NY,其包括人IL-11(缺少N端脯氨酸),平均分子量为40Kd的Y形状的PEG部分被连接至其N端。

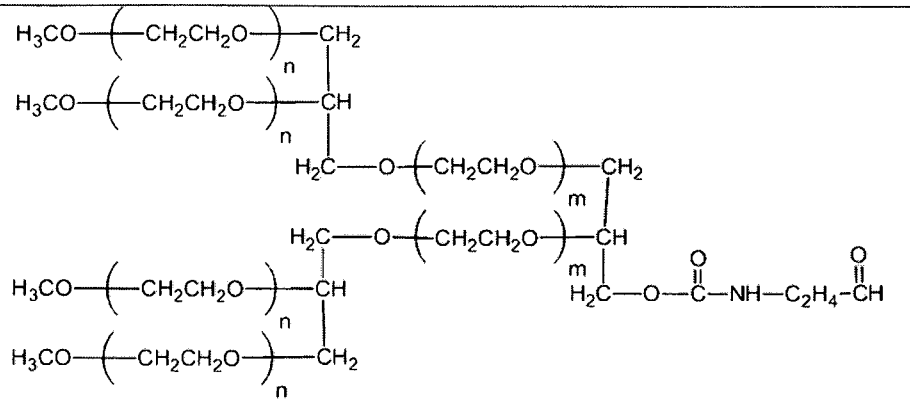
[0044] 在还进一步可选的方面,应当领会关于PEG部分的连接位置和/或连接种类,聚乙二醇化可以是混合的。因此,IL-11可以经受N端氨基酸处的随机的非共价聚乙二醇化和位点特异性聚乙二醇化,或经受N端氨基酸和内部氨基酸处不同的位点特异性聚乙二醇化。例如,并且最优选地,IL-11(或其任何修饰的形式)在N端氨基酸处可以被聚乙二醇化,并且除N端修饰之外还任选地在内部氨基酸处经由氮原子(例如,来自赖氨酸或组氨酸)被聚乙二醇化。

[0045] 例如,并且使用如图1中显示的截短的IL-11,本发明人使用如表1中显示的PEG试剂(其中n和m独立地是80和1000之间的整数,其取决于化合物的分子量)依照如制造商提供的和如下面更详细地进一步描述的实验方案进行聚乙二醇化。

[0046]

制造商/目录号	结构/分子大小
NOF/SUNBRIGHT ME-200AL	 20 KD
Jenkem/Y-PLA D-40K	 40 KD
NOF/SUNBRIGHT ME-120TS	 12 KD
NOF/SUNBRIGHT GL2-400TS	 40 KD
NOF/SUNBRIGHT GL2-200AL3	 20 KD
NOF/SUNBRIGHT ME-050TS	 5 KD

[0047]

NOF/SUNBRIGHT GL4-400AL3	 40 KD

[0048] 表1

[0049] 在聚乙二醇化截短的IL-11后，也如下面更详细地处理的，纯化如此获得的

[0050] 化合物，并且多种聚乙二醇化的IL-11分子具有如在表2中显示的下列名称：

[0051]

化合物代码	PEG 大小/KDa	PEG 的结构	缀合位点(键合)
I20NL	单个 20	直链	N 端(胺键)
I40NY	单个 40	Y 形状	N 端(胺键)
I12KL	单个 12	直链	N 端(酰胺键)
I40KY	单个 40	Y 形状	N 端(酰胺键)
I20NY	单个 20	Y 形状	N 端(胺键)
I20NL2	20×1~3	直链	N 端/赖氨酸或组氨酸(胺键)
I20NY2	20×1~3	Y 形状	N 端/赖氨酸或组氨酸(胺键)
I05KL4	5×4~5	直链	N 端/赖氨酸/组氨酸(酰胺键)
I40NX	单个 40	4-臂	N 端(胺键)

[0052] 表2

[0053] 最显著地，本发明人已经发现PEG部分的类型和连接的位点(和一定程度上，IL-11的序列)对体内生物学活性和稳定性具有出乎意料的和大幅度的影响。如根据下面的实验数据更明显的，尤其优选的聚乙二醇化使用单个Y形状的PEG部分在N端氨基酸处，特别地在IL-11被截短的地方。

[0054] 考虑的组合物

[0055] 基于本发明人发现了考虑的化合物的延长的生物学活性，通常考虑根据本发明主题的化合物可以配制用于治疗与缺乏IL-11相关联的多种疾病或特征在于对使用IL-11治疗有治疗响应的多种疾病。因此，在其它考虑的用途之中，本发明人尤其考虑包括考虑的化合物的药物组合物可以对治疗或预防下述疾病有效：(a)化疗诱发的血小板减少；(b)核事故/辐射诱发的骨和胃肠道(GI)损伤；(c)化疗诱发的骨和GI损害；(d)烧伤诱发的血小板减

少和GI损害；(e)血小板减少的其它病因；(f)GI损害的其它病因，包括炎性肠道疾病，如克罗恩病和溃疡性结肠炎，以及伪膜性结肠炎；(g)自由基诱发的肺损害；和/或(h)心血管疾病，其中考虑的药物组合物包括治疗有效量的考虑的化合物(或其药学上可接受的盐、水合物或前体药物)和药学上可接受的载体。例如，在本发明主题的一方面，考虑的组合物配制用于治疗化疗诱发的血小板减少或GI损害或辐射诱发的骨和胃肠道(GI)损害。可选地或额外地，还应当领会考虑的组合物可以被配制以诱发急性期蛋白质，和/或调节抗原-抗体反应。

[0056] 特别优选的是考虑的化合物被包括如此组合物中：其与一种或多种无毒的药学上可接受的载体一起配制。合适的药物组合物优选地配制用于注射或输注，或用于以固体或液体形式口服施用。因而，应当领会根据本发明主题的药物组合物可以使用多种途径——包括肠胃外地、口服地、腹膜内地和外用地——施用至人和其它动物(通常是哺乳动物)。

[0057] 例如，用于注射的适合的药物组合物优选地包括药学上可接受的无菌水性或非水性溶液、分散液、乳液或悬浮液，以及无菌粉末——用于在使用之前重构为无菌可注射溶液或分散液。合适的水性和非水性载体、稀释剂、溶剂或媒介的实例包括水、林格氏溶液、和等渗的氯化钠溶液、乙醇、多元醇(例如，甘油、丙二醇、聚乙二醇等)、和其合适的混合物、油、和可注射的有机酯(例如，油酸乙酯)。考虑的组合物还可以包含多种非活性成分，其包括防腐剂、润湿剂、乳化剂和/或分散剂。可以通过包含抗菌剂和/或抗真菌剂(例如，对羟基苯甲酸酯、苯酚、山梨酸、氯丁醇等)，以及通过穿过亚微米膜(例如，0.45μM或0.22μM孔径)过滤、高压灭菌或巴氏灭菌和辐射(例如γ或电子束)来确保无菌。在适当的情况下，可以包括渗透活性剂(例如，糖、氯化钠等)。虽然不限制本发明主题，但是用于注射的考虑的制剂典型地在3-9，更典型地6-8，和最典型地7.4+/-0.3的pH值范围中。当然，也应当认识到所有液体制剂可以以多种方式保存以促进长期贮存/储备。例如，考虑的稳定的方式包括使用冷冻干燥、喷雾干燥、结晶、(优选地，生物相容的或药学上可接受的)固相上吸附等的水/溶剂去除。

[0058] 可以使用多种途径施用根据本发明主题的组合物，所述途径包括口服地、肠胃外地、通过吸入、外用地、直肠地、鼻部地、或经由植入的储器，其中如本文使用的术语“肠胃外的”包括皮下、静脉内、肌肉内、关节内、滑膜内(intrasynovial)、鞘内、肝内、病灶内、和颅内施用(通常是注射或输注)。优选地，组合物经由注射施用，通常是静脉内地，并且更优选地皮下地。考虑的药物组合物还可以被外用施用，尤其是当治疗靶标包括通过外用施用容易接近的区域或器官——包括眼、皮肤、下肠道或在外科手术期间暴露的区域——的疾病时。存在本领域已知的众多外用制剂，并且所有这样的制剂均被认为适于在本文使用。

[0059] 关于组合物中考虑的化合物的量，应当认识到具体数量将通常取决于特定制剂和期望的目的。因此，应当认识到考虑的化合物的量将显著地变化。然而，通常优选的是化合物以对在体外和/或体内递送治疗效果有效的最小量存在。

[0060] 因而，在最优选的实施方式中，考虑的化合物将以大约0.1μg/ml至大约100mg/ml之间，更典型地大约10μg/ml至大约10mg/ml之间，和最典型地大约5μg/ml至大约100μg/ml之间的量存在。关于剂量单位，通常考虑以对取得期望的治疗效果有效的剂量施用考虑的化合物，通常是10-100μg/kg，并且更优选地30-70μg/kg。然而，可选的剂量单位可以在0.1-10μg/kg、或50-80μg/kg、或80-120μg/kg、或120-200μg/kg之间，或甚至更高。从不同的角度

来看，应当领会考虑的制剂的单次使用单位可以包括大约0.3mg至3.0mg之间的聚乙二醇化的IL-11，或大约3mg至7mg之间的聚乙二醇化的IL-11，或大约7mg至10mg之间的聚乙二醇化的IL-11(最典型地，具有7-9×10^6U/mg的比活性)。除非上下文另外相反规定，本文陈述的所有范围均应当解释为包括其端点，并且开放式范围应当解释为包括商业上实践的值。类似地，除非上下文相反规定，所有列举的值应当被视为包括中间值。

[0061] 此外，应当注意考虑的制剂可以包括一种或多种额外的药学活性剂，其可以存在于相同的制剂中，或可以使得单独可获得(以不同类型的制剂或相同类型的制剂)，或可以作为试剂盒售卖。例如，合适的额外药学活性剂包括多种类固醇(例如，皮质类固醇)、刺激骨髓中血小板产生的药剂(例如，Li_2CO_3、叶酸等)、抗体、止痛剂和抗炎剂。

[0062] 考虑的用途

[0063] 考虑的化合物作为治疗剂对单独或联合使用治疗下述疾病可以是特别有用的：(a)核事故/辐射诱发的骨和胃肠道(GI)损害；(b)化疗诱发的骨和GI损害；(c)烧伤诱发的血小板减少和GI损害；(d)血小板减少的其它病因；(e)GI损害的其它病因，包括炎性肠道疾病，如克罗恩病和溃疡性结肠炎，以及伪膜性结肠炎；(f)自由基诱发的肺损害；和(g)心血管疾病。

[0064] 因此，本发明人还考虑本文提供的化合物用于制造治疗下述疾病的药物中的用途：(a)核事故/辐射诱发的骨和GI损害；(b)化疗诱发的骨和GI损害；(c)烧伤诱发的血小板减少和GI损害；(d)血小板减少的其它病因；(e)GI损害的其它病因，包括炎性肠道疾病，如克罗恩病和溃疡性结肠炎，以及伪膜性结肠炎；(f)自由基诱发的肺损害；和(g)心血管疾病。

[0065] 从另一个角度来看，本发明人还考虑治疗有需要的人中的下述疾病的方法：(a)核事故/辐射诱发的骨和GI损害；(b)化疗诱发的骨和GI损害；(c)烧伤诱发的血小板减少和GI损害；(d)血小板减少的其它病因；(e)GI损害的其它病因，包括炎性肠道疾病，如克罗恩病和溃疡性结肠炎，以及伪膜性结肠炎；(f)自由基诱发的肺损害；和(g)心血管疾病，其中以治疗有效剂量施用考虑的化合物。

[0066] 实验和实验数据

[0067] 材料：纯化批次(bulk)的重组人IL-11——其源自酵母——由Hangzhou Jiuyuan Gene Engineering Company(批号20121005/1006/1007/1008)提供。7TD1鼠杂交瘤细胞系获取自DSMZ(No.ACC23)。Paraplatin®注射液(通用名称：卡铂)10mg/mL(批次：5A03935)由Bristol-Myers Squibb Company制造。测序级的胰蛋白酶——由牛胰腺改造——(目录号11418025001)购自Roche diagnostics。小鼠IL-11受体α获取自MyBioSource,Inc.(目录号MBS553276)。CellTiter 96®水性非放射性细胞增殖试验(MTS)(目录号G5430)购自Promega，用于7TD1细胞试验。用于人IL-11的DuoSet ELISA显影试剂盒购自R&D Systems Inc.(目录号DY218)。纯化树脂-MacroCap SP(产品代码17-5440-01)获取自GE Healthcare Life Sciences。精确的Tris-甘氨酸8-16%聚丙烯酰胺凝胶购自Thermo Scientific。HPLC用途的三氟乙酸(目录号302031)和乙腈(目录号34967)购自Sigma-Aldrich。

[0068] 目录号为SUNBRIGHT®ME-120TS、ME-200AL、GL2-400TS、ME-050TS、GL4-400AL3、GL2-200AL3的多种形式的单官能PEG购自NOF Corporation，并且Y-PALD-40K购自Jenkem Technology USA。PEG试剂的分子结构在上面显示在表1中。

[0069] I12KL/I40KY/I05KL4的制备：在大约8的pH下，5mg/mL蛋白质被引入有1至2倍摩尔比的各自的PEG试剂(用于I12KL的NOF/SUNBRIGHT ME-120TS；用于I40KY的SUNBRIGHT GL2-400TS)和50mM $NaHCO_3$的混合物。使用相同的方式制备I05KL4(PEG试剂：NOF/SUNBRIGHT ME-120TS)，除了PEG与蛋白质的摩尔比被增加到12倍。反应混合物在室温下培育2小时，接着使用2mM甘氨酸猝灭。使用如下的层析纯化程序分离聚乙二醇化的产物。PEG分子通过酰胺键合被连接至蛋白质。

[0070] I20NL/I40NY/I20NY/I20NL2/I20NY2/I40NX的制备：5mg/mL蛋白质被引入有1至2倍摩尔比的各自的PEG试剂(用于I20NL和I20NL2的NOF/SUNBRIGHT ME-200AL；用于I40NY的Jenkem/Y-PLAD-40；用于I20NY和I20NY2的NOF/SUNBRIGHT GL2-200AL3；用于I40NX的NOF/SUNBRIGHT GL4-400AL3)、10mM氰基硼氢化钠和50mM NaH_2PO_4的混合物。对于缀合至两个位点上，PEG的摩尔比增加到3.5至5.5倍。pH被调节至大约4.5-5.0。反应混合物在室温下培育24小时，接着使用2mM甘氨酸猝灭。PEG分子通过更稳定的胺键合被连接至蛋白质。使用如下的层析纯化分离聚乙二醇化的产物。

[0071] 层析纯化：使用1M醋酸将蛋白质溶液的pH调节至4-5，接着离心或过滤以去除颗粒。引入四体积的水。对于包含超过20KDa的PEG的缀合物：将蛋白质溶液装载至MacroCap SP柱(1×6cm)上，该柱使用包含20mM醋酸钠pH 5的缓冲液A进行平衡。使用包含20mM醋酸钠pH 5和1M NaCl的缓冲液B的梯度或逐步洗脱来洗脱蛋白质。对于包含低于20KDa的PEG的缀合物：将蛋白质溶液装载至MacroCap SP柱(1×6cm)上，该柱使用包含20mM磷酸钠pH 7的缓冲液A进行平衡。使用包含20mM磷酸钠pH 7和1M NaCl的缓冲液B的梯度或逐步洗脱来洗脱蛋白质。可以在图2中看到如在SDS PAGE凝胶中分析的典型的最终产物。在此，左泳道装载有分子量标记，并且多种聚乙二醇化形式的IL-11被装载入其余泳道。注意，I40NY运行到超过100Kd的表观分子量，大于估计的60Kd的分子量，这可能由于其PEG部分的Y形状。在进一步特别优选的方面，考虑的化合物的纯化作为一步纯化过程进行，其在下游放大试验(scale-up)中提供了增加的优势。

[0072] 通过RP-HPLC的纯度检查：每种PEGamer的含量通过采用与二极管阵列检测器——来自Thermo Scientific的UltiMate 3000快速分离LC系统—连接的UPLC的反相(RP)层析进行分析。层析程序使用如下进行：柱：Acquity C18，1.7μm，2.1×150mm，300Å孔径，装备有保护筒(guard cartridge)；流动相A：50％(v/v)乙腈中的0.1％(v/v)TFA；流动相B：95％(v/v)乙腈中的0.1％(v/v)TFA；流速：0.4ml/min；柱温度：65℃；检测：214nm；注入20μg，并且如下面的表3中运行梯度。

[0073]

时间(min)	A%	B%
0	100	0
2	100	0
9.9	80	20
9.95	0	100
11.3	0	100
11.31	100	0
17.5	100	0

[0074] 表3

[0075] 蛋白质含量的测定:通过UV/Vis微板(microplate)和比色池分光光度计——来自Thermo Scientific的Multiskan GO——测定蛋白质含量。消光系数的单位是$M^{-1}cm^{-1}$,在280nm下在水中测量为17,990。可选地,使用对于0.1%(1mg/ml)溶液0.944的吸光度值,通过在280nm波长下的紫外线光谱学直接测定蛋白质浓度。使用在280nm下的吸光度的蛋白质定量测量芳香族氨基酸比如色氨酸和酪氨酸的吸光度,而未检测PEG部分。结果,本文陈述的按重量计的蛋白质浓度并不包含PEG分子。

[0076] 健康大鼠中的药代动力学(PK)研究:在以100-150μg/kg的给药水平通过静脉内或皮下途径单剂量施用考虑的化合物之后,在3只雄性SD大鼠中实施体内操纵。在肝素管中以众多时间点采集血液样品,接着进行血浆分离并且在-20℃下储存。通过用于人IL-11的DuoSet ELISA试剂盒(R&D Systems Inc.目录号DY218)测定免疫反应性IL-11在血浆样品中的浓度。使用非隔室(non-compartment)模型通过WinNonlin 5.3软件产生药代动力学的参数。

[0077] 健康大鼠中的药效动力学(PD)研究:在100-150μg/kg的给药强度下,使用静脉内或皮下施用各自的考虑的化合物,在4只雄性SD大鼠中实施药效动力学评估。在肝素管中以众多时间点采集血液样品,接着进行血浆分离并且在-20℃下储存。在Cell-DYN 3500血液学分析仪上实施血细胞计数。

[0078] 骨髓抑制性大鼠中的药效动力学(PD)研究:在第0天利用静脉内施用40mg/kg下的卡铂诱发骨髓抑制,在4只雄性SD大鼠中实施药效动力学评估。在第1天以150μg/kg皮下注射考虑的化合物。在肝素管中以众多时间点采集血液样品,接着进行血浆分离并且在-20℃下储存。在Cell-DYN 3500血液学分析仪上实施血细胞计数。

[0079] 胰蛋白酶作图(mapping):在包含2mg/mL蛋白质和1/50(W/W)胰蛋白酶的50mM Tris pH 8.3缓冲液中制备反应溶液。在室温下培育6小时,接着添加等体积的0.2%TFA(三氟乙酸)溶液。在注入至HPLC上之前,通过离心去除任何颗粒物质。使用如下实施层析程序:柱:Zorbax 300SB-C8,2.1×150mm,5μm,300Å孔径;流动相A:0.1%(v/v)TFA;流动相B:95%(v/v)乙腈中的0.1%(v/v)TFA;流速:0.2ml/min;检测:214nm;注入10μg,并且如下面的表4中运行梯度。

[0080]

时间(min)	A%	B%
0	100	0
3	100	0
8	95	5
45	55	45
45.1	0	100

[0081]

52	0	100
52.1	100	0
65	100	0

[0082] 表4

[0083] 使用HPLC结合MS光谱法(Thermo LCQ Advantage)实施蛋白水解肽的鉴定。

[0084] 在一些实施方式中，用于描述和要求保护本发明的某些实施方式的表达成分的数量、特性比如浓度、反应条件等的数字被理解为在一些情况下由术语“大约”修饰。因此，在一些实施方式中，在撰写的说明书和所附的权利要求中陈述的数值参数是近似值，其可以取决于具体实施方式试图获得的期望的性质而变化。在一些实施方式中，应当根据报道的有效数字和通过应用普通的舍入技术解释数值参数。尽管陈述本发明的一些实施方式的大范围的数值范围和参数是近似值，但在具体实例中陈述的数值被尽可能精确地报道。在本发明的一些实施方式中提供的数值可以包含必然由在它们各自的测试测量中发现的标准偏差造成的某些误差。

[0085] 虽然通常已知PEG可以赋予其缀合物血浆稳定性，但是不可预测什么连接类型、链长度和分子结构将关于治疗效果和/或药理学参数产生特定的结果。在第一个实例中，在正常大鼠中关于血浆稳定性调查多种PEG大小的截短的IL-11缀合物。在静脉内施用之后，发现所观察的未聚乙二醇化的IL-11的血浆半衰期与其聚乙二醇化的对应物的那些(3.5-13.7hr)相比非常短，小于10分钟。在聚乙二醇化的对应物之中，较高分子量的PEG以下列顺序赋予较大的血浆稳定性：I40KY(13.7hr)～I40NY(8.5hr)>I20NL(3.8hr)～I12KL(3.5hr)。图3图解了在单次静脉内施用后多种形式的聚乙二醇化的IL-11的血浆浓度。每种样品以100μg/kg的剂量在大鼠中给药。可以推断出在此实例中较大或较长的PEG链导致较长的血清半衰期。

[0086] 在第二个实例中，在100μg/kg下的单次静脉内施用后，按照静脉内途径测量血小板增量，使用健康SD大鼠实施药效动力学评估。如由图4可见，40-KD缀合物(I40NY和I40KY)诱发比I20NL(50%)更大的血小板增量(60-75%)，其中Y代表Y形状的PEG，并且L代表直链形PEG。结果还表明多个缀合(I05KL4，四个位点上缀合5-KD PEG)比N端上的单个长的PEG链较不有效，这是由于具有较短的PEG缀合物的多个仅具有大约25%的血小板增量的有限功效。在此实例中推断出单个位点上较长的PEG链在血小板诱发的方面导致较高的功效。这似乎与聚乙二醇化对重组人生长激素的影响相反。

[0087] 在第三个实例中，通过胰蛋白酶作图结合蛋白水解肽的LC/MS鉴定来调查缀合位

点。图5描绘了IL-11(未缀合的)、I40NY和I40KY的胰蛋白酶图谱,并且下面的表5提供了胰蛋白酶肽。

[0088]

质量	位置	肽#	肽序列
773.9	1-8	T1	GPPPGPPR
669.7	9-14	T2	VSPDPR
1217.4	15-25	T3	AELDSTVLLTR
774.9	26-32	T4	SLLADTR
798.9	33-39	T5	QLAAQLR
261.3	40-41	T6	DK
3319.8	42-74	T7	FPADGDHNLDSLPTLAM SAG ALGALQLPGVLTR
287.4	75-76	T8	LR
950.1	77-84	T9	ADLLSYLR
839.0	85-90	T10	HVQWLR
174.2	91-91	T11	R
618.7	92-98	T12	AGGSSLK
1327.5	99-110	T13	TLEPELGTLQAR
402.5	111-113	T14	LDR
400.5	114-116	T15	LLR
174.2	117-117	T16	R
860.1	118-124	T17	LQLLMSR
2600.0	125-150	T18	LALPQPPPDPPAPPLAPP SS AWGGIR
1914.2	151-168	T19	AAHAILGGLHLTLDWA VR
655.9	169-174	T20	GLLLLK
275.3	175-176	T21	TR
131.2	177-177	T22	L

[0089] 表5

[0090] 在此可见对应于T1肽的峰在I40NY和I40KY的胰蛋白酶图谱中明显降低。这指示具有PEG的两种缀合物均被连接至T1肽上,其中N端胺是用于化学缀合的唯一位点。结果,I40NY和I40KY均是N端连接的,然而,仅仅差别于化学键合,其中I40NY是胺键,而I40KY是酰胺键。显著地,I40NY和I40KY二者经由静脉内施用在血清半衰期和血小板诱发中展示出类似的效果。

[0091] 由于胺键比酰胺键更稳定,并且对于使用还原性胺化的选择性聚乙二醇化,单-聚

乙二醇化的产物的产率更均匀，因此在后续研究中，在血小板产生的功效方面调查了多种N端缀合物，并且在健康大鼠中评价其各自经由皮下施用的相关的副作用。图6描绘了六种聚乙二醇化的IL-11缀合物以150μg/kg下的皮下施用在大鼠中的药效动力学研究的结果。每天施用IL-11持续连续14天，而聚乙二醇化的IL-11每周注射一次。PEG形状可以影响缀合物的功能。具体地，非直链形PEG分子与其直链对应物相比赋予更好的血浆稳定性和更大的效力。如图6中展示的，I20NY与I20NL(46-55%)相比诱发更大的血小板增量(58-70%)，其中Y代表Y形状的PEG并且L代表直链形PEG。这些结果表明Y形状的PEG与相同分子量的直链形式相比具有更大的效力。然而，I40NY(Y形状)和I40NX(4-臂梳子形状)在血小板产生中是相当的，如二者均增加高至约65-70%，这表明当PEG大小为大约或超过40KD时PEG形状的影响变得饱和。显著地，相同PEG长度的双聚乙二醇化降低了体内功效，如I20NL2(直链PEG至两个位点上)和I20NY2(Y形状的PEG至两个位点上)与它们的单个聚乙二醇化的对应物相比具有较低的血小板产生。因此，推断出在多种N端缀合的IL-11之中，I40NY、I40NX和I20NY发挥了较高的功效。而且，注意到不管缀合位点的数目如何，较小的PEG缀合物比如20-KD PEG的第二次施用的效果不知为何被下调。结果，I40NY和I40NX是出乎意料有效的化合物，其具有期望的生物学性质和相对适度的不利效应(尤其是特别是血浆膨胀)。而且，生物学数据进一步表明如此修饰的IL-11化合物可以较不频繁地施用，并且最优选地每周两次、每周一次、或甚至更少。在考虑的化合物用于治疗大群体中的血小板减少(例如，暴露于放射暴露)时，这样的安排是特别相关的。

[0092] 本发明人还在健康大鼠中研究了与IL-11缀合物相关联的副作用。血细胞比容状态通常被用作评估IL-11的临床使用中的副作用的标记，这是因为患者可能由于血浆膨胀经历稀释性贫血。在动物研究中，IL-11以150μg/kg皮下施用持续连续14天，而聚乙二醇化的IL-11以相同的剂量每周注射一次。如图7中显示的，所有药物均导致减小的血细胞比容，但是发现I40NY与其余的聚乙二醇化的缀合物相比具有更小的降低，同时维持更高的活性。当在相同的图表中与其它使用缀合的和未缀合的IL-11的个体动物实验比较时，给药I40NY减轻稀释性贫血是更突出的。在图8中建立了血小板产生和如通过血细胞比容的降低显现的副作用之间的相关性，当将多种修饰的和未修饰的IL-11标绘在图表中时，这表明了副作用随着增加的和剂量依赖的功效而加剧的趋势。不同剂量下的I40NY明显位于趋势的右上侧上，这指示在相当的功效的基础上与一些其它化合物和未修饰的IL-11相比更小的血浆膨胀。在产品表征的方面，本发明人表征了优选化合物I40NY的物理化学和药理学性质。

[0093] IL-11缀合物的基于细胞的试验：使用7TD1细胞系(DSMZ,Germany)在细胞增殖试验中测试缀合的IL-11的生物学活性。简言之，在存在2μg/mL鼠IL-11受体(MyBioSource,USA,MBS553276)的情况下，4000个细胞/孔的7TD1细胞响应于不同的IL-11浓度在37℃下在5%CO_2的潮湿气氛中生长两天(Biochem.J.,318:489-495)。在添加MTS后，通过针对y轴上的IL-11浓度在y轴上标绘490nm的吸光度，通过使用GraphPad软件Prism 6拟合S形剂量反应曲线来确定剂量反应曲线的EC50。在动物研究前，使用7TD1细胞系在细胞增殖试验中测试新合成的缀合物的生物学活性。并非所有聚乙二醇化制备均给予类似的产物，并且实际的产物形成取决于被缀合的IL-11的氨基酸残基，以及使用的PEG分子的大小和形状。7TD1细胞响应于不同的缀合物浓度生长。在添加显影剂——其化学信号与细胞数目具有线性关系——后，在ELISA酶标仪中读取490nm下的吸光度。结果显示在图9中。

[0094] 由于PEG部分的空间位阻，与未聚乙二醇化的IL-11相比，所有缀合物在基于细胞的试验中均预期地显示出具有以下列效力顺序的生物活性的降低：IL-11(100%)>I20NL、I20NY(均大约16%)>I40NY、I40NX(均11%)>I40KY(6%)>I20NY2(3%)。显著地，空间干扰在确定聚乙二醇化的缀合物的生物活性中是主导因素，并且可以看作缀合物——其整个PEG部分大于20KDa——的生物活性的剧烈减小。根据一些基于细胞的研究，存在显示如下的报道：当与在其它连接位点处的那些缀合物比较时，IL-11分子的非核心区比如N端序列处的小的碳水化合物连接增强生物学活性(J.Biol.Chem.Vol 286,No.10,pp 8085-8093)，其与由IL-11的N端序列处的PEG分子较少降低的生物活性一致。虽然I40NY的体外生物活性仅保留天然IL-11的大约11%，但是体内功效被有益地影响并且其不能由体外生物活性数据预测。下面的表6示意性地说明了与未修饰的IL-11相比多种化合物的生物活性比。

[0095]

鉴定	与未修饰的IL-11的生物活性比
IL-11	1
I20NL	0.13
I20NY	0.16
I20NY2	0.03
I40NY	0.11
I40NX	0.11
I40KY	0.06

[0096] 表6

[0097] 使用PEG的蛋白质的化学修饰是已建立的技术并且已经应用于生物制药产业以增强蛋白质的溶解度和物理-化学稳定性。虽然此化学反应易于进行，但是其通常导致包含PEGamer和位置异构体的不同的聚乙二醇化形式的复杂混合物。采用多个层析纯化步骤以高回收分离产物。为了研发出在成本和产率的方面商业上可行的过程，许多因素比如蛋白质浓度、PEG的质量、蛋白质/PEG比率、反应温度、和缓冲液pH、以及纯化过程需要被优化。

[0098] 通过在形成稳定的胺键的胺上利用Y形状的聚乙二醇链的缀合构建I40NY，其具有由缀合化学过程(PEG部分中醛偶联基团的还原性胺化)驱动的对N端胺的相对高的选择性。另一方面，在形成对应的酰胺键的易接近的胺上、在pH 8下使用官能化的NHS试剂缀合I40KY。更具体地，I40NY是在酸性条件下使用位点特异性反应产生的单-聚乙二醇化的IL-11，这是因为官能化的醛对N端α-胺是很大选择性的，其pKa与其它亲核体相比更低。在缀合反应中调查PEG与蛋白质比、反应浓度、pH和动力学。在存在10mM氰基硼氢化钠的情况下，反应以大约0.05-0.5mL体积的小规模在室温(22-27℃)下进行24小时。通过RP-UPLC测定研究中每个反应的产率。对于选择的反应使用不同的pH，最佳缀合产率在pH 4.5-5.5下。此外，本发明人注意到反应物的浓度在产物产率中起重要作用，并且发现在大于5mg/mL的浓度下与IL-11缀合是最佳的。同样，调查在存在还原剂的情况下、在室温下使用5mg/mL蛋白质的反应的PEG与蛋白质比和缀合动力学，并且表明PEG与蛋白质的最佳摩尔比是2，并且反应延长至16小时足以单-聚乙二醇化。

[0099] 聚乙二醇化的蛋白质的纯化通常采用大规模制备的离子交换层析。然而，当常规的离子交换器以少至1mg/mL树脂的装载容量装载有反应产物时，无法达到令人满意的分离

度(resolution)来将单-聚乙二醇化的与低-聚乙二醇化的分开。此树脂的低容量通常限制其用于较大规模生产的应用。为了以高纯度分离N端单-聚乙二醇化的IL-11，测试多种阳离子交换树脂。显著地，高孔隙度树脂(例如，来自GE Healthcare Life Sciences的MacroCap SP)提供了高容量以及保持的分离度，并且在高装载条件下提供了高纯度和产率的单-聚乙二醇化的靶标。在存在10mM氰基硼氢化钠的情况下，以400mg批量大小的IL-11——其在包含2摩尔比的醛活化的40-KD Y形状的PEG试剂的磷酸钠pH 4.5-5缓冲液中以5mg/mL制备——证明纯化过程。通过添加2M甘氨酸猝灭反应溶液，接着以4×体积的去离子水进行稀释。在通过0.2μm膜过滤后，得到的粗制品被装载至MacroCap SP柱(2.6(直径)×10(高度)cm，大约7.5mg/mL树脂的装载容量)上。装料后，使用超过10倍柱体积的20mM醋酸钠pH 5缓冲液清洗柱，接着另外使用超过20倍柱体积的包含0.1M NaCl的20mM醋酸钠pH 5缓冲液清洗柱。然后，使用包含0.3M NaCl的20mM醋酸钠pH 5缓冲液洗脱产物。分离的I40NY的总产率是26.6%。通过SDS-PAGE和反相HPLC检查I40NY的产物纯度，并且图10显示了在银染色的SDS-PAGE凝胶上I40NY的纯度，其具有如在泳道上方指示的I40NY的数量。如在图11中展示的层析图，单-聚乙二醇化的IL-11的纯度大于93%，如通过C18-HPLC测定的。

[0100] 为了测定经由皮下途径的I40NY的药代动力学参数，3只雄性SD大鼠经由单次皮下施用注射有0.15mg/kg聚乙二醇化的IL-11。图12展示了在单次皮下施用后大鼠中免疫反应性IL-11的血浆浓度。缀合的IL-11的血浆浓度在大约12hr处达到最大水平，并且在施用后超过50小时保持有效。相反，重组人IL-11在大约2hr处达到最大浓度，并且快速地从循环血流清除，这是由于血浆中消除半衰期是大1.3hr。经由皮下途径的I40NY的药代动力学参数在下面的表7中被总结。

[0101]

	单位	I40NY	IL-11
$T_{1/2}$，终末半衰期	hr	18.6	1.1
T_{max}，至最大浓度的时间	hr	12	2
C_{max}，最大血浆浓度	ng/mL	142	147
AUC_{all}，曲线下面积	Hr*ng/mL	3947	700
AUC_{inf}，至无限的曲线下面积	Hr*ng/mL	4421	701
Vz，相对分布体积	mL/kg	909	347
Cl，相对清除率	mL/hr/Kg	33.9	214
MRT，平均滞留期	hr	27.2	3.7

[0102] 表7

[0103] 使用圆二色谱调查I40NY的二级结构。在远UV区中所分析的圆二色谱的色谱图中，本发明人证明I40NY维持与其未缀合的对应物相同的二级结构，如由在图13中叠加的两个光谱可见的。而且，通过测量它们的二级结构响应于热应激的改变(平均残基椭圆率)，通过圆二色谱证明I40NY的热稳定性。图14指示响应于温度增量I40NY的结构改变较小。

[0104] 还在卡铂处理的大鼠中证明I40NY在骨髓抑制性大鼠中的效力。雄性SD大鼠经由静脉内施用注射有40mg/kg的卡铂，以诱发骨髓的功能受损，其导致血小板减少。以相同的0.15mg/kg剂量使用每天注射(连续7天)IL-11或单个剂量的I40NY的医疗干预在24小时的卡铂处理后立即皮下施用。血小板水平展示在图15中。在不治疗的情况下，对象经历大约两

天的严重的血小板减少(小于1/3的正常血小板计数),这表明未治疗的具有危及生命的内出血的高风险。IL-11治疗的功效是边际的,这是由于每天给药的最低点非常靠近严重的血小板减少的阈值。相反,单个剂量的I40NY不仅预防发生严重的血小板减少,而且加速血小板水平的恢复,如血小板计数恢复至初始数目比其它两组早1.3天。

[0105] 同时,还在骨髓抑制性模型中调查如在血细胞比容的降低上显现的副作用。在图16中,与未治疗组相比,使用IL-11的治疗以快速的方式引起血细胞比容降低。然而,单个剂量的I40NY缓和最低点,这表明与使用IL-11的每天给药相比较不加剧的副作用。因而,应当领会I40NY已经证明在预防由化疗诱发的严重的血小板减少中有效,同时可改善血浆膨胀的综合征。

[0106] I40NY和另一种形式的聚乙二醇化的IL-11(如在US8133480中描述的,数据未显示)之间进一步的比较数据揭示与在'480专利中描述的其它形式的聚乙二醇化的IL-11相比,考虑的化合物并且尤其是I20NY和I40NY具有显著增强的体内效力和降低的副作用的综合征。

[0107] 对本领域技术人员显而易见的是,除已经描述的那些之外,更多修改也是可能的,而不背离本文发明性的概念。因此,除所附权利要求的范围以外,本发明主题不被限制。而且,在解释说明书和权利要求二者中,所有术语应当以与上下文一致的最大可能的方式解释。具体而言,术语"包括"和"包含"应当解释为以非排他性方式指要素、组件或步骤,其指示所提及的要素、组件或步骤可以存在、利用或与其它未明确提及的要素、组件或步骤一起组合。在说明书所附权利要求指选自A、B、C…和N的某物中的至少一种时,该文本应当解释为仅需要来自该组中的一个要素,而非A加N、或B加N等。

序列表

<110> 南沙生物制品(香港)

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<210> 1
[0108] <211> 177
<212> PRT
<213> 智人

<220>
<221> 肽
<222> (1)..(177)
<223> 缺少 N 端 P 的人 IL-11

<400> 1

Gly Pro Pro Pro Gly Pro Pro Arg Val Ser Pro Asp Pro Arg Ala Glu
1 5 10 15

Leu Asp Ser Thr Val Leu Leu Thr Arg Ser Leu Leu Ala Asp Thr Arg
20 25 30

Gln Leu Ala Ala Gln Leu Arg Asp Lys Phe Pro Ala Asp Gly Asp His
35 40 45

Asn Leu Asp Ser Leu Pro Thr Leu Ala Met Ser Ala Gly Ala Leu Gly
50 55 60

Ala Leu Gln Leu Pro Gly Val Leu Thr Arg Leu Arg Ala Asp Leu Leu
65 70 75 80

Ser Tyr Leu Arg His Val Gln Trp Leu Arg Arg Ala Gly Gly Ser Ser
85 90 95

Leu Lys Thr Leu Glu Pro Glu Leu Gly Thr Leu Gln Ala Arg Leu Asp
100 105 110

[0109]

Arg Leu Leu Arg Arg Leu Gln Leu Leu Met Ser Arg Leu Ala Leu Pro
115 120 125

Gln Pro Pro Pro Asp Pro Pro Ala Pro Pro Leu Ala Pro Pro Ser Ser
130 135 140

Ala Trp Gly Gly Ile Arg Ala Ala His Ala Ile Leu Gly Gly Leu His
145 150 155 160

Leu Thr Leu Asp Trp Ala Val Arg Gly Leu Leu Leu Leu Lys Thr Arg
165 170 175

Leu

10 20 30 40 50 60

GPPPGPPRVS PDPRAELDST VLLTRSLLAD TRQLAAQLRD KFPADGDHNL DSLPTLAMSA

螺旋 A

70 80 90 100 110 120

GALGALQLPG VLTRLRADLL SYLRHVQWLR RAGGSSLKTL EPELGTLQAR LDRLLRRLQL

螺旋B 螺旋 C

130 140 150 160 170

LMSRLALPQP PPDPPAPPLA PPSSAWGGIR AAHAILGGLH LTLDWAVRGL LLLKTRL

螺旋 D

图1

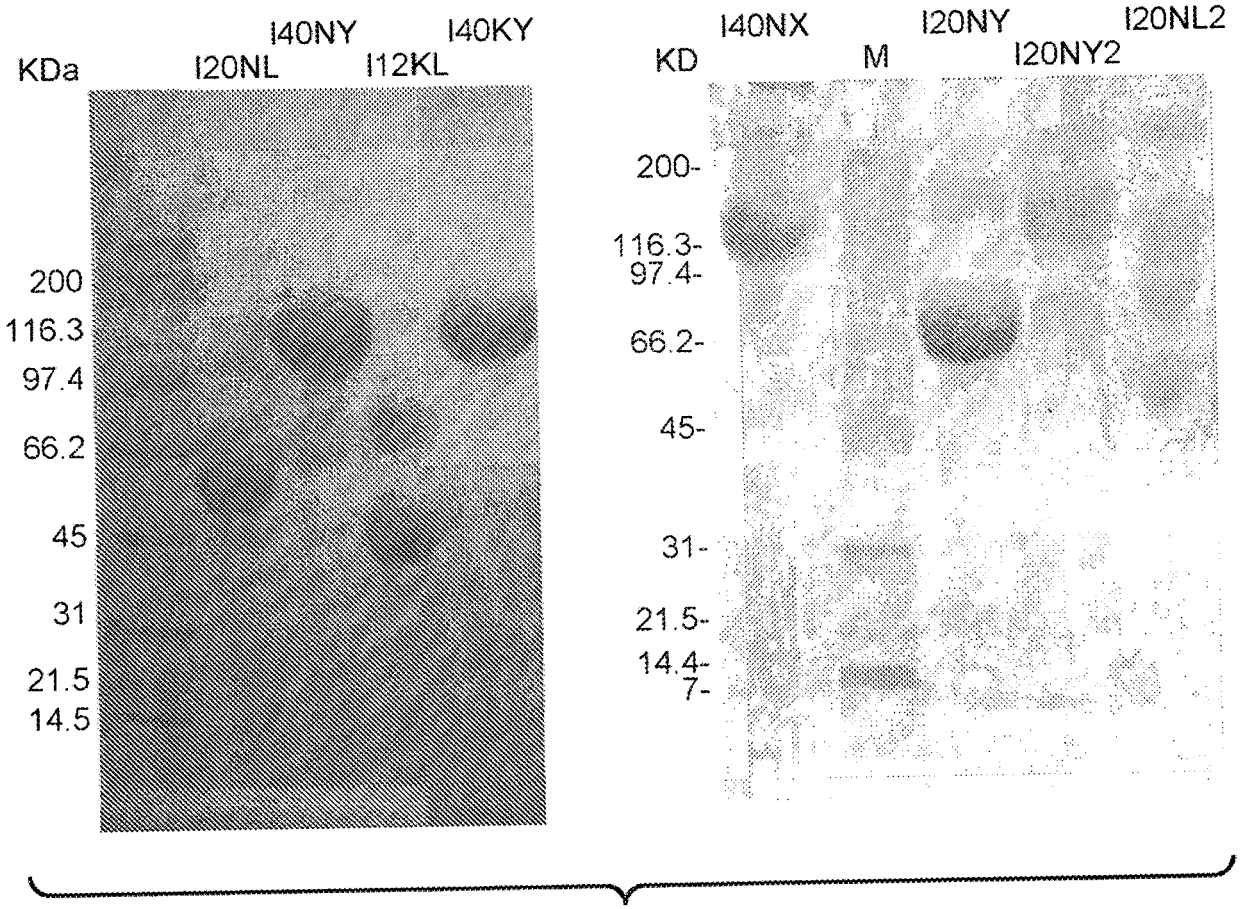


图2

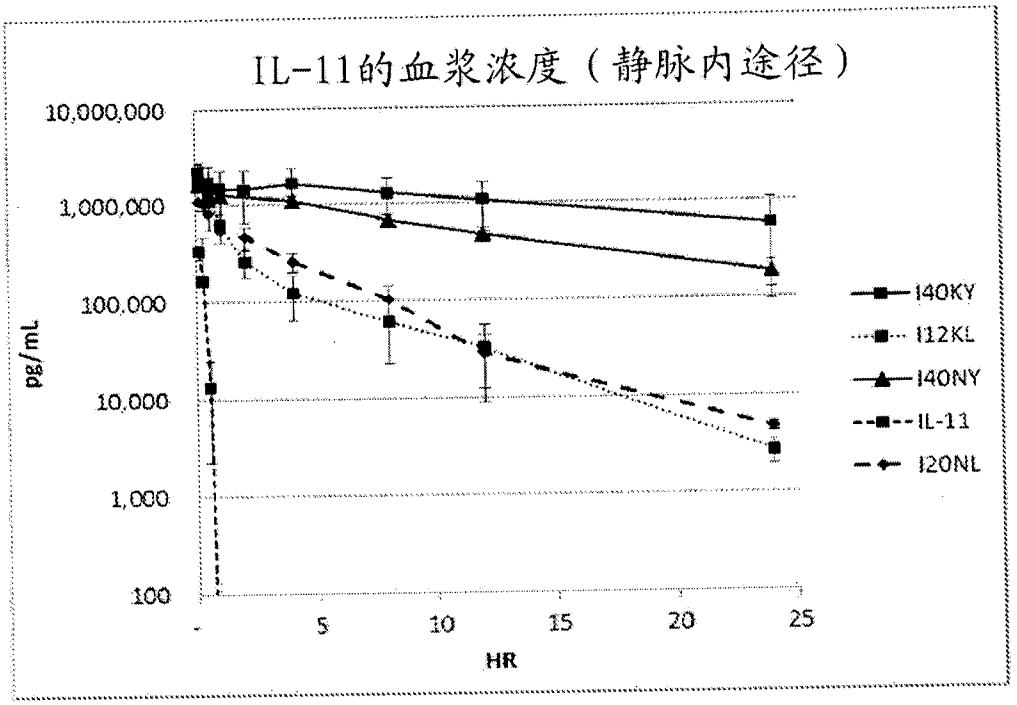

IL-11的血浆浓度（静脉内途径）
10,000,000
1,000,000
100,000
10,000
1,000
100
pg/mL
-
5
10
15
20
25
HR
I40KY
I12KL
I40NY
IL-11
I20NL

图3

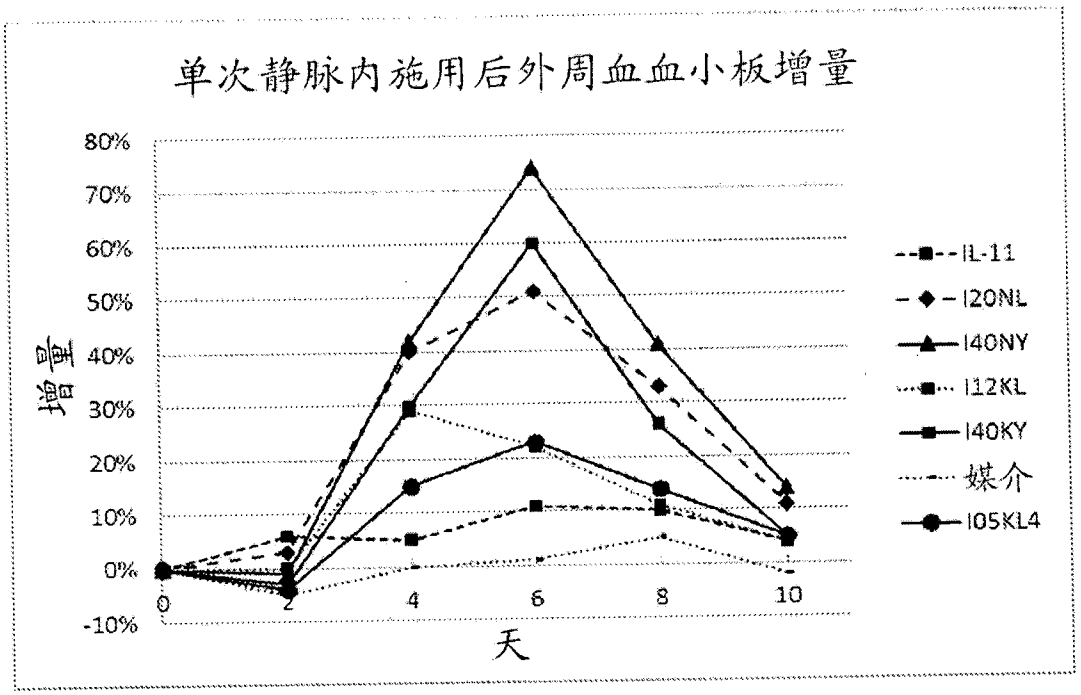

单次静脉内施用后外周血血小板增量
80%
70%
60%
50%
40%
30%
20%
10%
0%
-10%
增量
0
2
4
6
8
10
天
IL-11
I20NL
I40NY
I12KL
I40KY
媒介
I05KL4

图4

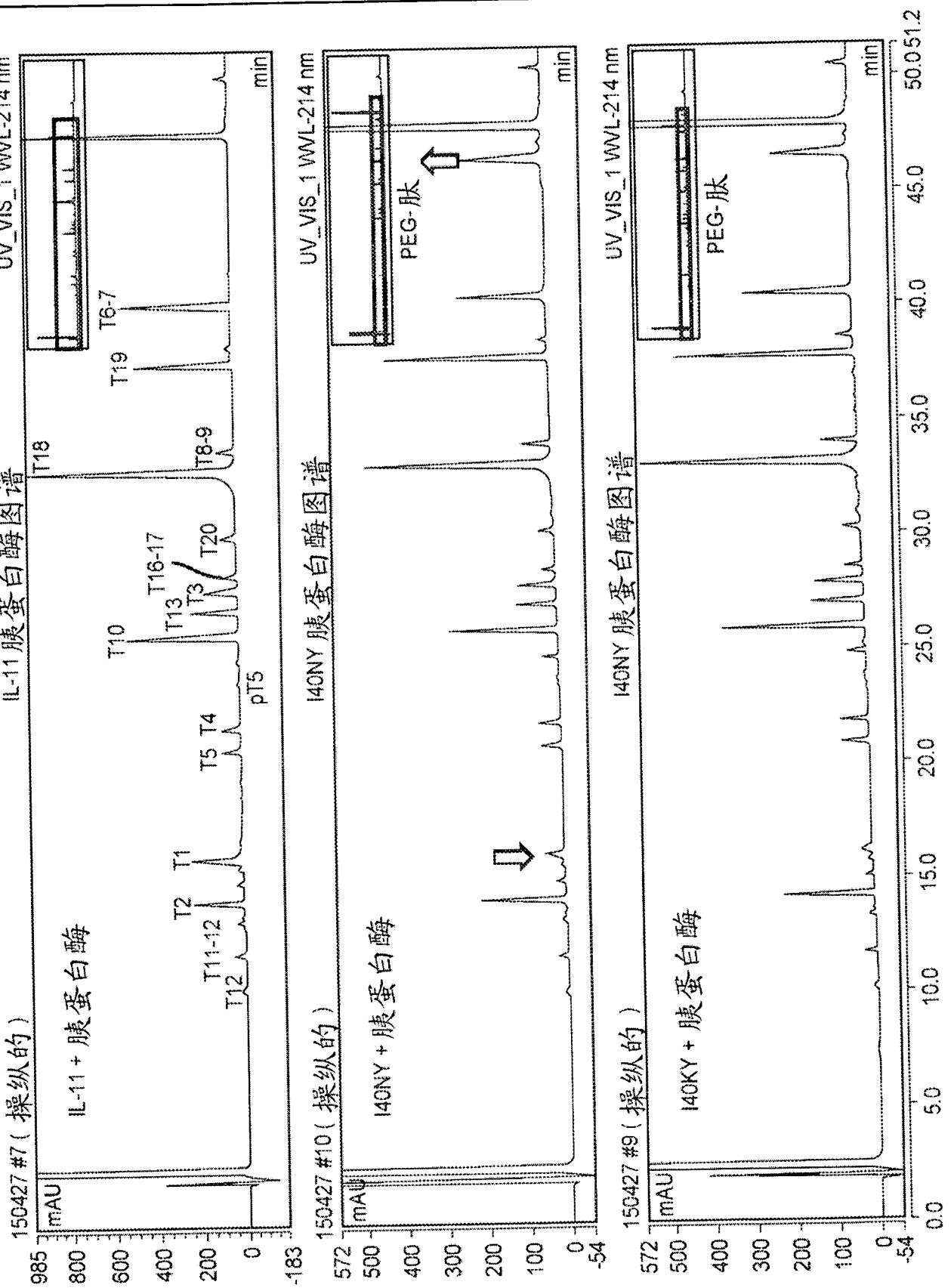

UV_VIS_1 WVL-214 nm
IL-11 胰蛋白酶图谱
150427 #7 (操纵的)
IL-11 + 胰蛋白酶
mAU
985
800
600
400
200
0
-183
min
T18
T19
T6-7
T10
T16-17
T13
T3
T20
T8-9
T2
T1
T11-12
T12
T5
T4
pT5
UV_VIS_1 WVL-214 nm
I40NY 胰蛋白酶图谱
150427 #10 (操纵的)
I40NY + 胰蛋白酶
mAU
572
500
400
300
200
100
0
-54
min
PEG-肽
UV_VIS_1 WVL-214 nm
I40NY 胰蛋白酶图谱
150427 #9 (操纵的)
I40KY + 胰蛋白酶
mAU
572
500
400
300
200
100
0
-54
min
PEG-肽
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0
51.2

图5

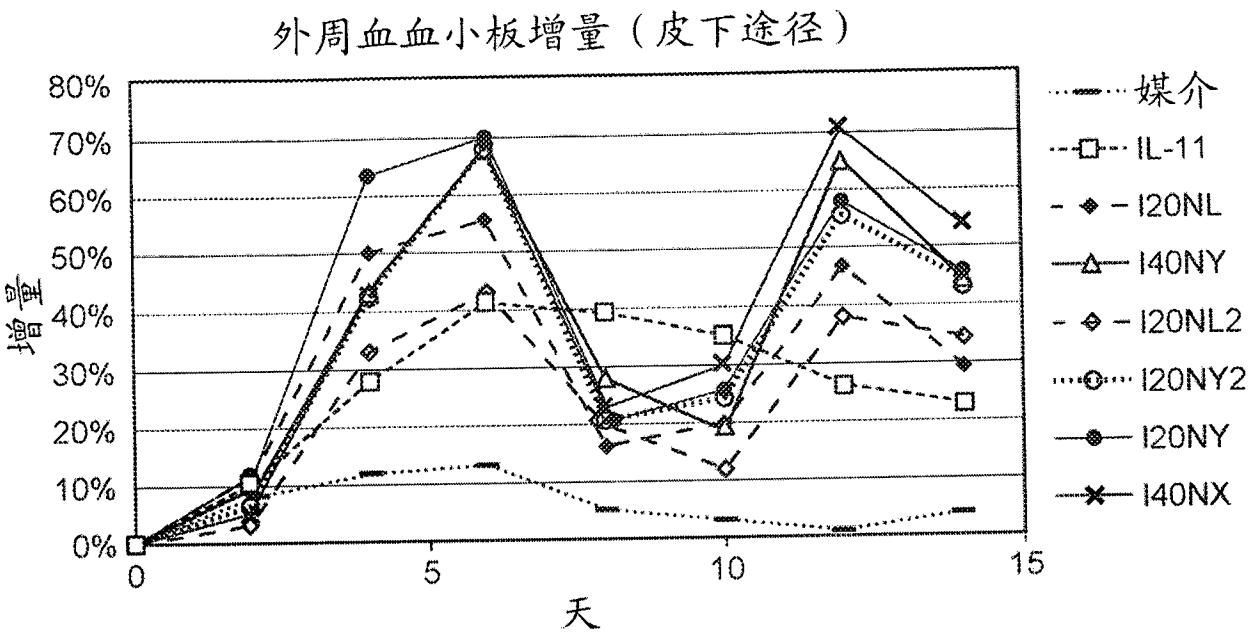

外周血血小板增量（皮下途径）
80%
70%
60%
50%
40%
30%
20%
10%
0%
增量
0
5
10
15
天
媒介
IL-11
I20NL
I40NY
I20NL2
I20NY2
I20NY
I40NX

图6

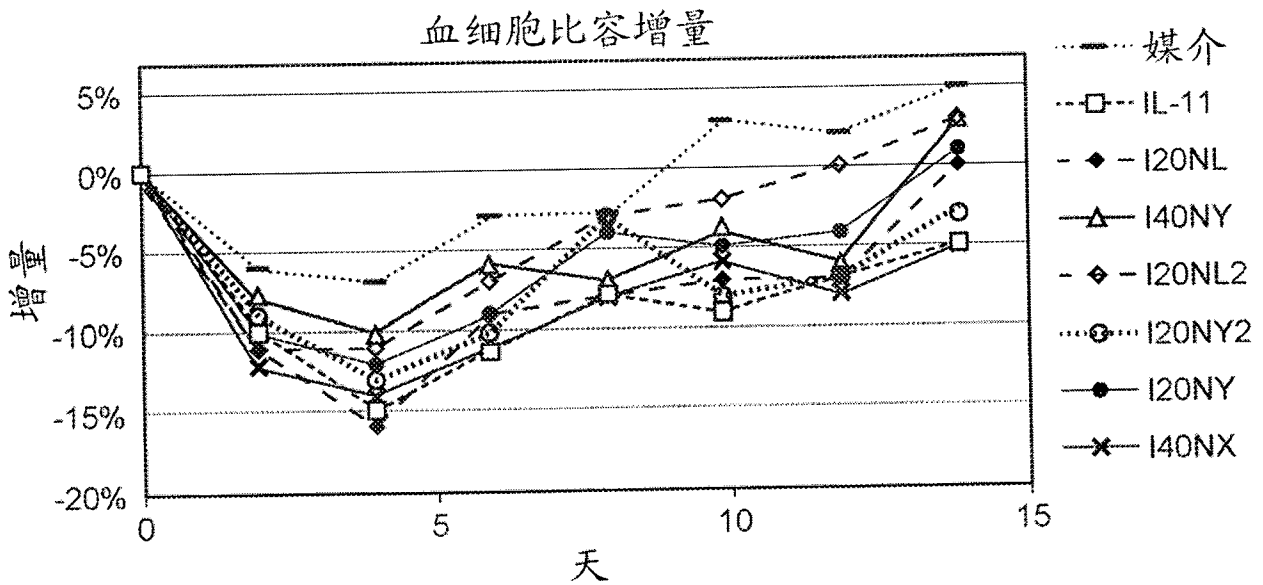

血细胞比容增量
5%
0%
-5%
-10%
-15%
-20%
增量
0
5
10
15
天
媒介
IL-11
I20NL
I40NY
I20NL2
I20NY2
I20NY
I40NX

图7

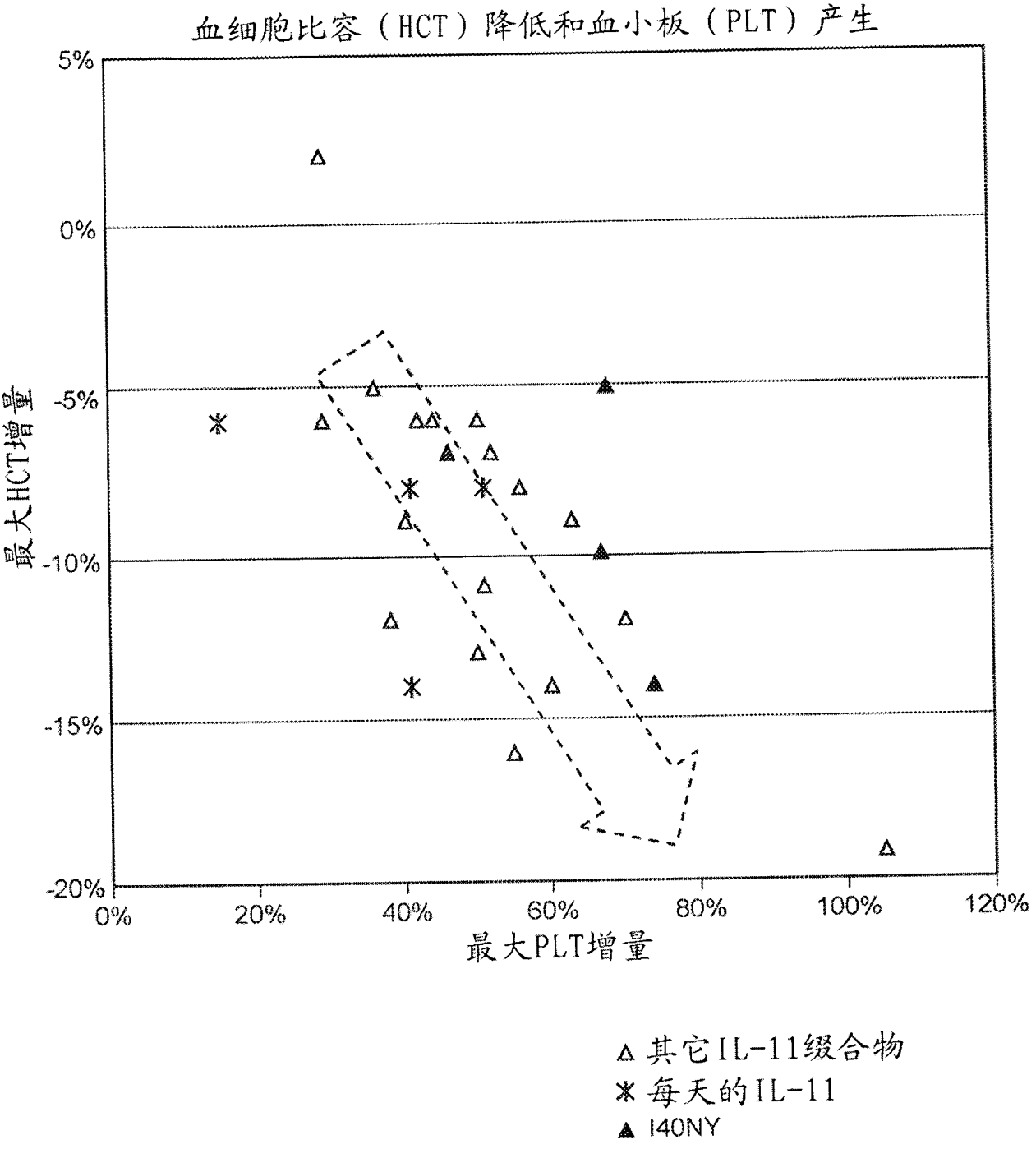
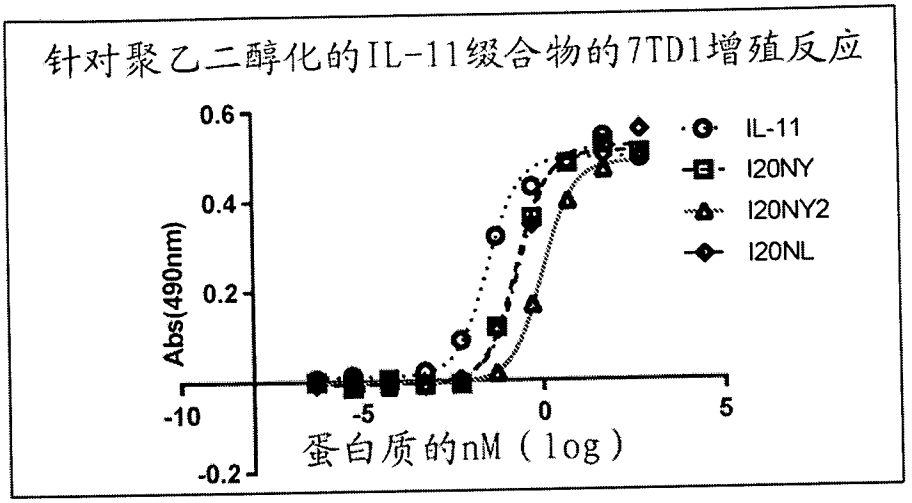
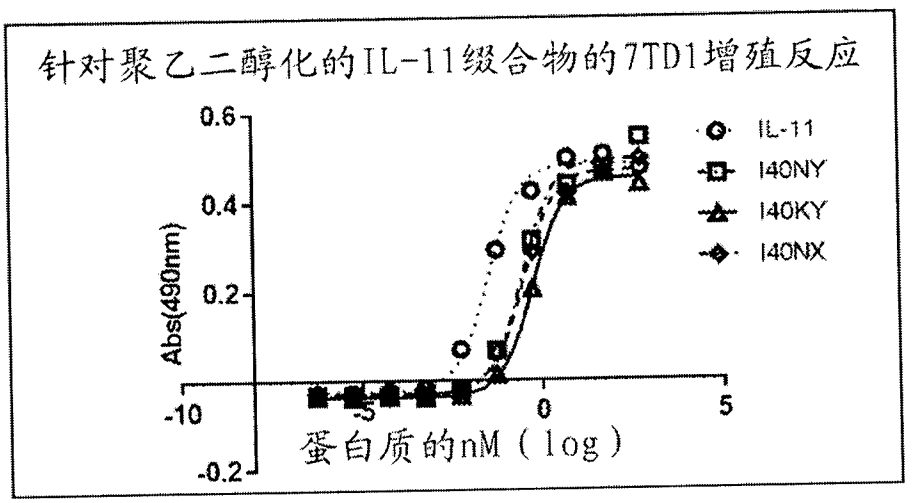

血细胞比容（HCT）降低和血小板（PLT）产生
5%
0%
-5%
-10%
-15%
-20%
最大HCT增量
0%
20%
40%
60%
80%
100%
120%
最大PLT增量
△ 其它IL-11缀合物
✱ 每天的IL-11
▲ I40NY

图8



	IL-11	I20NY	I20NY2	I20NL
EC50(pM)	30.49	191.60	1012.0	239.80
决定系数	0.9918	0.9979	0.9988	0.9954
与未修饰的IL-11的效力比	1	0.16	0.03	0.13



	IL-11	I40NY	I40KY	I40NX
EC50(pM)	30.80	277.20	536.40	292.90
决定系数	0.9957	0.9926	0.9991	0.9968
与未修饰的IL-11的效力比	1	0.11	0.06	0.11

图9

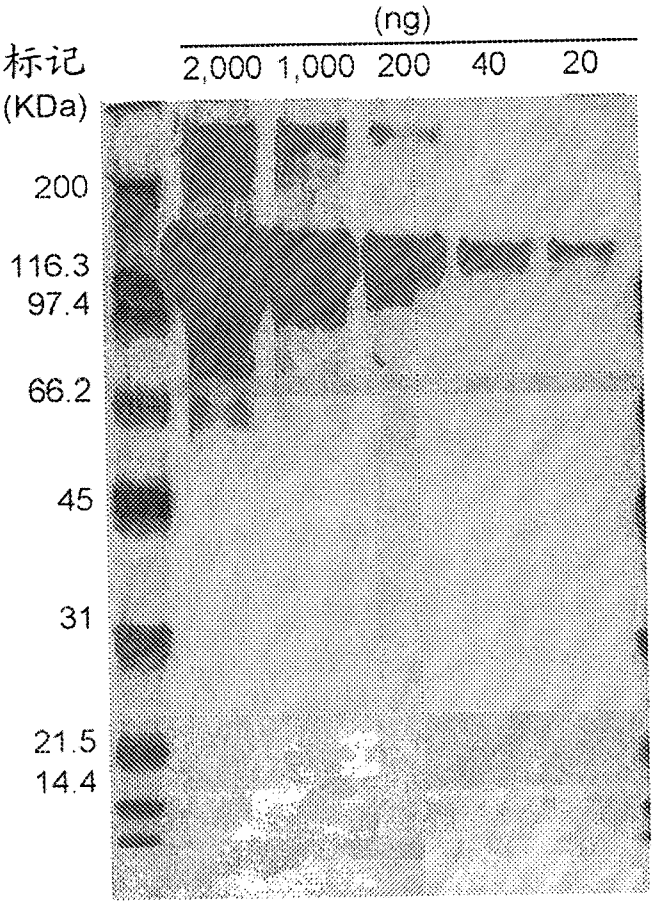

(ng)
标记
2,000 1,000 200 40 20
(KDa)
200
116.3
97.4
66.2
45
31
21.5
14.4

图10

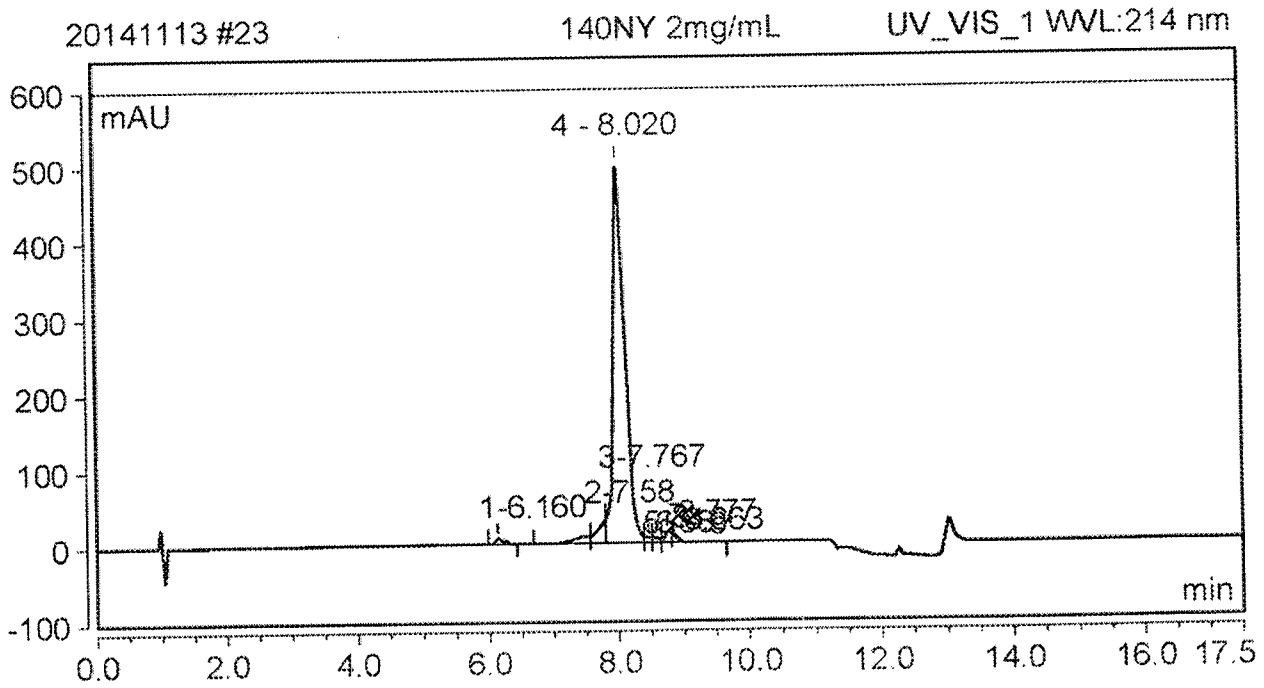

20141113 #23
140NY 2mg/mL
UV_VIS_1 WVL:214 nm
mAU
600
500
400
300
200
100
0
-100
4 - 8.020
3-7.767
2-7.58
1-6.160
min
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
17.5

图11

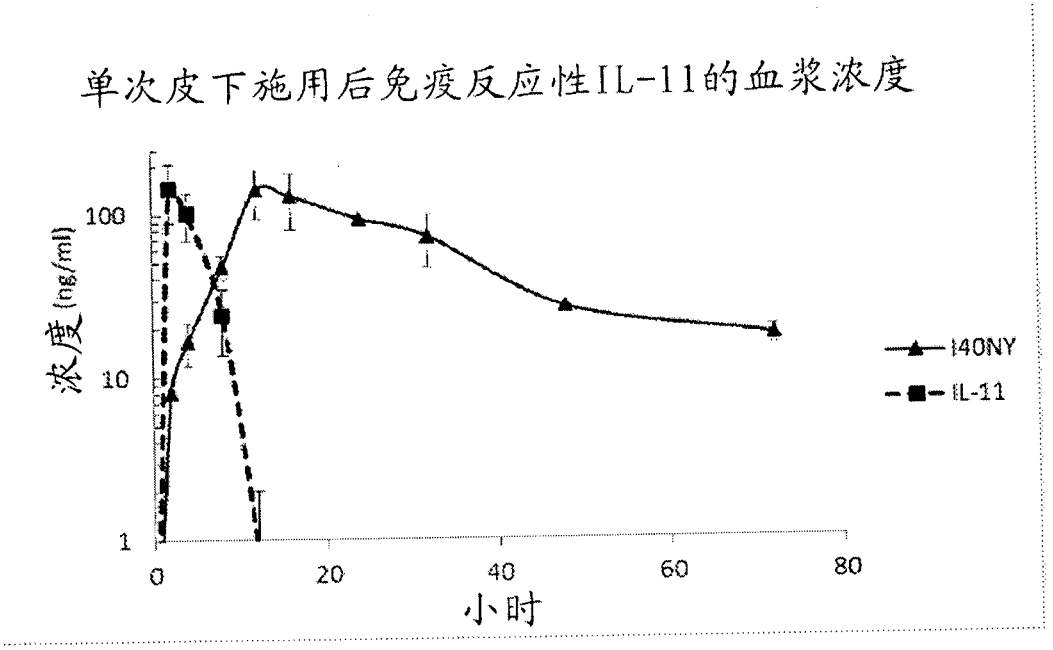

单次皮下施用后免疫反应性IL-11的血浆浓度
浓度(ng/ml)
100
10
1
0
20
40
60
80
小时
I40NY
IL-11

图12

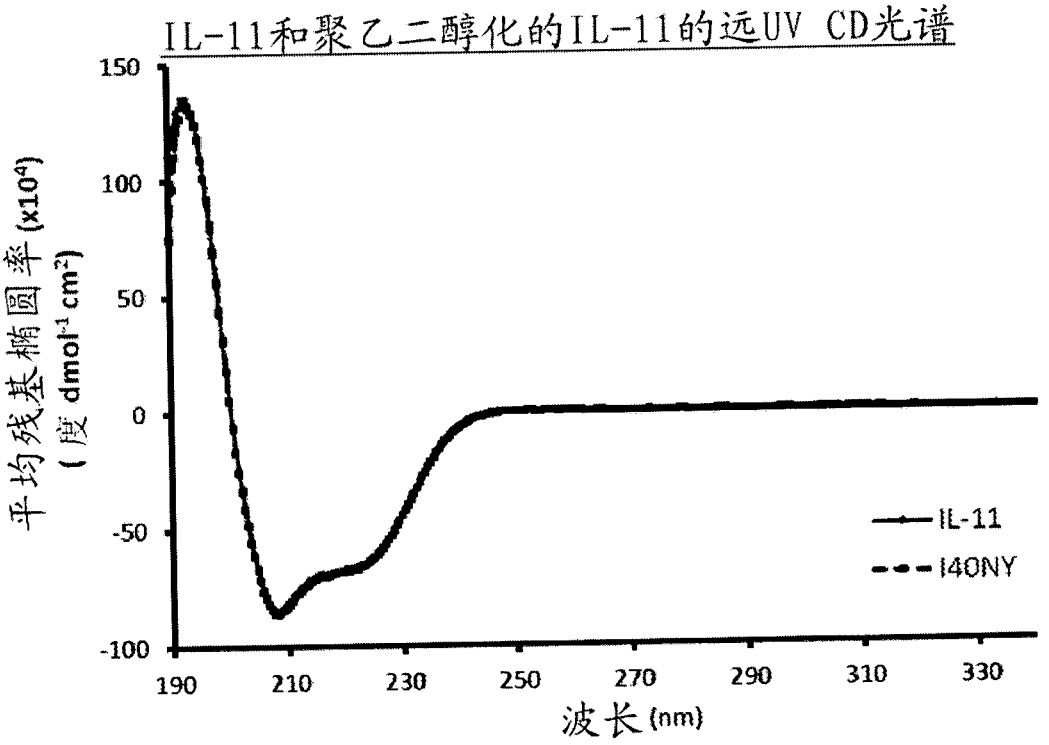

IL-11和聚乙二醇化的IL-11的远UV CD光谱
平均残基椭圆率 (x10⁴)
(度 dmol⁻¹ cm²)
150
100
50
0
-50
-100
190
210
230
250
270
290
310
330
波长(nm)
IL-11
I40NY

图13

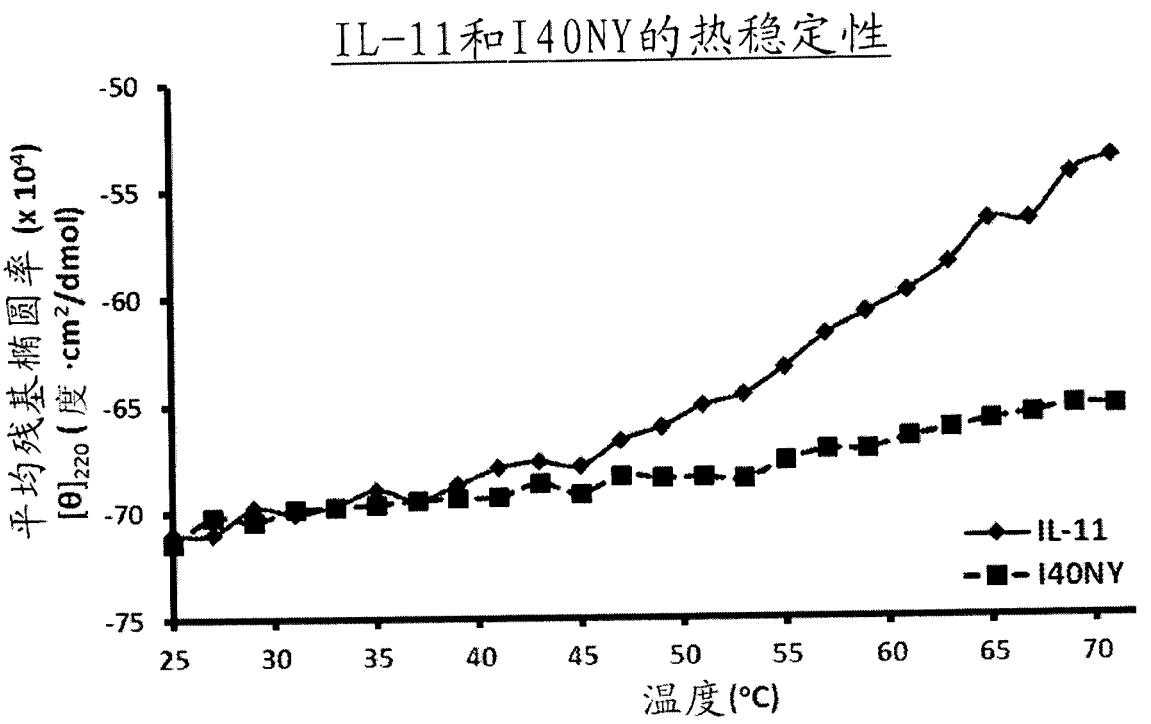

IL-11和I40NY的热稳定性
平均残基椭圆率 (x 10⁴)
[θ]220 (度·cm²/dmol)
-50
-55
-60
-65
-70
-75
25
30
35
40
45
50
55
60
65
70
温度(°C)
IL-11
I40NY

图14

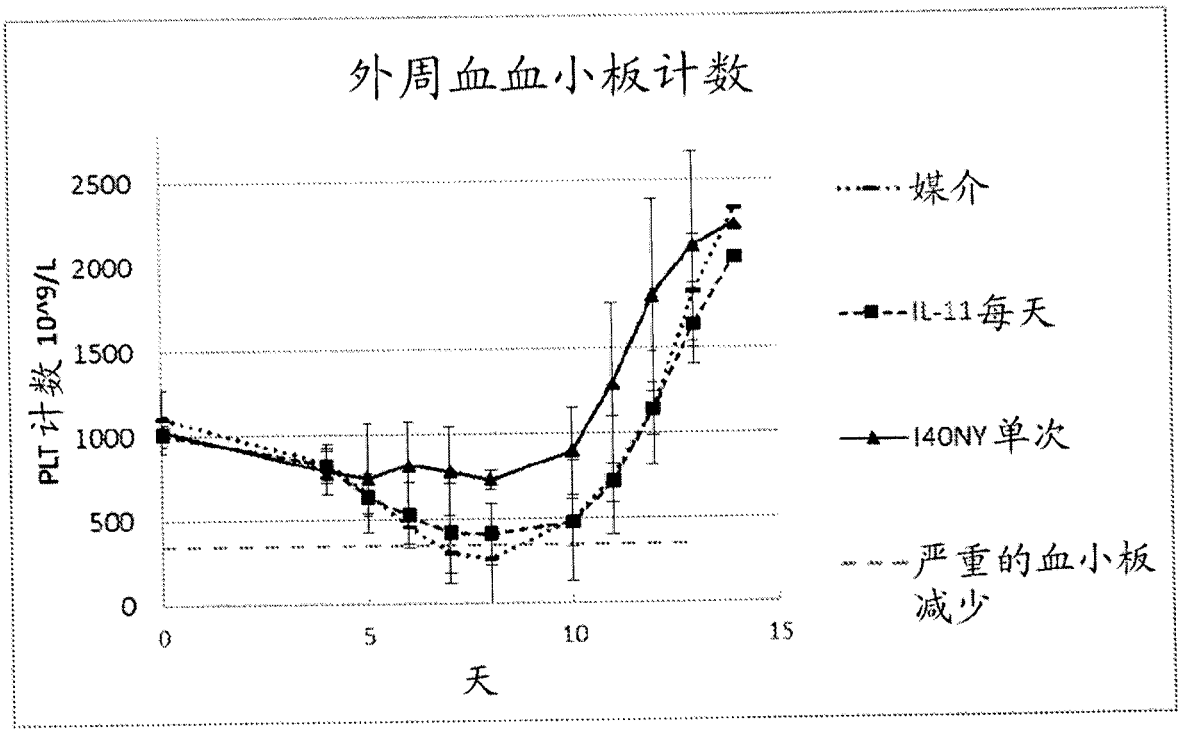

外周血血小板计数
PLT计数 10^9/L
2500
2000
1500
1000
500
0
0
5
10
15
天
媒介
IL-11每天
I40NY单次
严重的血小板减少

图15

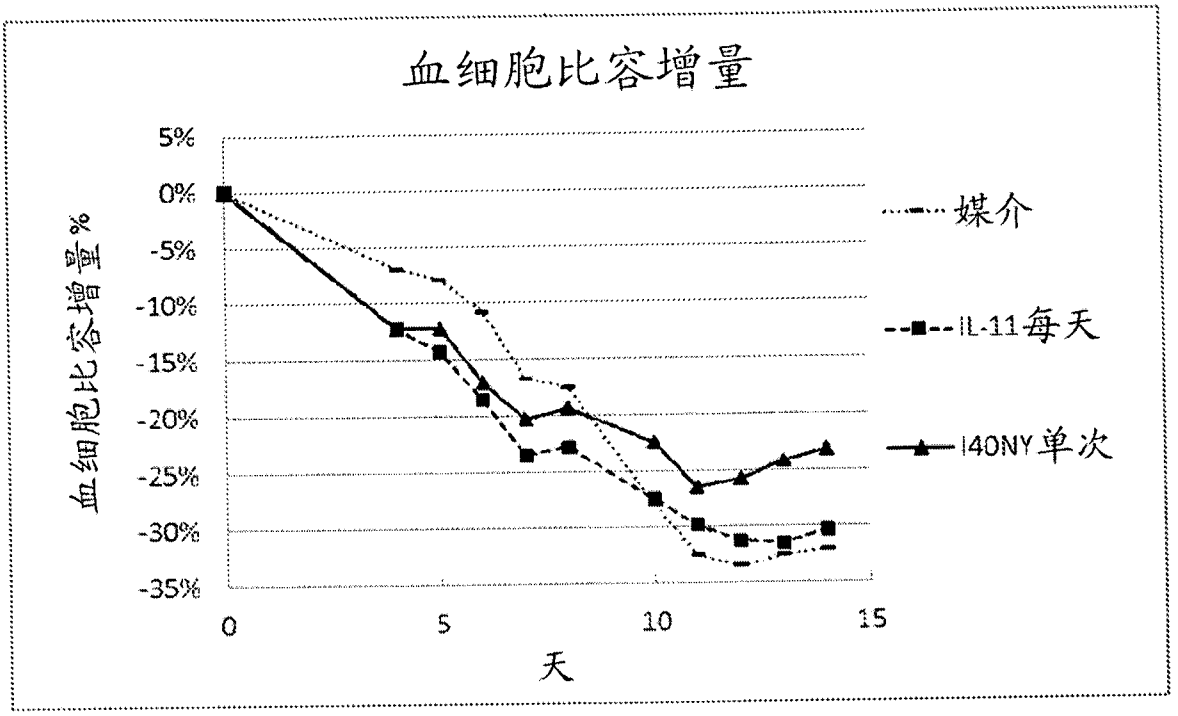

血细胞比容增量
血细胞比容增量%
5%
0%
-5%
-10%
-15%
-20%
-25%
-30%
-35%
0
5
10
15
天
媒介
IL-11每天
I40NY单次

图16

Abstract

COMPOSITIONS AND METHODS FOR PEGYLATED IL-11

Compositions and methods are presented in which recombinant IL-11 is PEGylated to achieve improved half-life in serum while having desirable therapeutic activity and presenting less side-effects. Most preferably, the IL-11 is an N-terminally truncated human or humanized IL-11 and has a 20Kd or 40Kd branched PEG moiety, Y- or comb- shaped in particular, coupled to the N-terminal amino group. Such compounds are characterized by substantially increased stability in serum and sustained biological activity while exhibiting significantly reduced plasma expansion.

摘要

聚乙二醇化的IL-11的组合物和方法

提供了组合物和方法，其中重组IL-11被聚乙二醇化以取得改进的血清中的半衰期，同时具有期望的治疗活性并呈现更少的副作用。最优选地，IL-11是N端截短的人或人源化IL-11并且具有20Kd或40Kd支化的PEG部分，特别是Y形状的或梳子形状的，其偶联至N端氨基。这样的化合物的特征在于大幅度增加的血清中的稳定性和持久的生物学活性，同时展示显著降低的血浆膨胀。