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(54) **Title:** CYTOKINE MUTEINS

(57) **Abstract:** The present invention relates generally to the treatment of an interleukin-11 (IL-11)-mediated condition. More particularly, the present invention provides the use of modified forms of IL-11 which modulate IL-11 signaling in the treatment of IL-11-mediated conditions.

## CYTOKINE MUTEINS

### APPLICATION DATA

5 [0001] This application is associated with and claims priority from U.S. Provisional Patent Application No. 61/000,576, filed on 26 October, 2007, the entire contents of which are incorporated herein by reference.

### FIELD

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[0002] The present invention relates generally to the treatment of an interleukin-11 (IL-11)-mediated condition. More particularly, the present invention provides the use of modified forms of IL-11 which modulate IL-11 signaling in the treatment of IL-11-mediated conditions.

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### BACKGROUND

[0003] Bibliographic details of references provided in the subject specification are listed at the end of the specification.

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[0004] Reference to any prior art is not, and should not be taken as an acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in any country.

25 [0005] Interleukin-11 (IL-11) is a member of the IL-6 family of cytokines which includes IL-6, viral IL-6 (vIL-6), leukemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1), cardiotrophin-like cytokine/cytokine-like factor-1 (CLC/CLF), IL-27 and neuropoietin (NP). IL-11 is able to stimulate the growth and differentiation of various lineages of hematopoietic cells, either alone or in  
30 synergy with other cytokines. IL-11 is also able to stimulate megakaryopoiesis and

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platelet production, and is used clinically to prevent chemotherapy-induced thrombocytopenia (Tepler *et al*, *Blood* 87(9):3607-3614, 1996) and is currently being assessed as a new approach to the treatment of chemotherapy-induced gastrointestinal mucositis (Herrlinger *et al*, *Am J Gastroenterol* 101(4):793-797, 2006). IL-11 has also  
5 been suggested as being of benefit in arthritis and inflammatory bowel disease.

[0006] IL-11 also exerts a variety of biological activities outside the hematopoietic system. It is a regulator of osteoclast development and believed to be a regulator of bone metabolism (Girasole *et al*, *J clin Invest* 93:1516-1524, 1994; Hughes *et al*, *Calcif tissue*  
10 *Int* 53:362-364, 1993; Heymann and Rousselle, *Cytokine* 12(10):1455-1468, 2000). IL-11 is expressed at high levels in cells of the CNS (Du *et al*, *J Cell Physiol* 168:362-372, 1996) and stimulates the survival and proliferation of neuronal progenitor cells (Mehler *et al*, *nature* 362:62-65, 1993). In female mice, IL-11 is essential for successful embryo implantation (Dimitriadeis *et al*, *Mol Hum Reprod*. 6(10):907-914, 2000; Robb *et al*, *Nat*  
15 *Med* 4:303-308, 1998; Bilinski *et al*, *Genes Dev* 12:2234-2243, 1998) and the expression pattern of IL-11 and its receptors during the menstrual cycle suggests a similar role in humans. Other non-hematopoietic activities of IL-11 include inhibition of adipogenesis (Ohsumi *et al*, *FEBS Lett* 288:13-16, 1991; Ohsumi *et al*, *Biochem Mol Biol Int* 32:705-712, 1994), induction of a febrile response (Lopez-Valpuesta *et al*, *Neruopharmacology*  
20 33:989-994, 1994), modulation of extracellular matrix metabolism (Maier *et al*, *J Biol chem.* 268:21527-21532, 1993), stimulation of acute-phase reactants (Baumann and Schendel, *J Biol Chem* 266:20424020427 1991), and proposed pro- and anti-inflammatory roles (Trepicchio *et al*, *J Clin Invest* 104:1527-1537, 1999; Redlich *et al*, *J Immunol* 157:1705-1710, 1996).

25

[0007] IL-11 has also been suggested as a potential therapeutic agent in various other inflammatory disorders including radiation-induced lung damage (Redlich *et al*, *supra* 1996), sepsis (Chang *et al*, *Blood Cells Mol Dis* 22(1):57-67, 1996) and psoriasis (Trepicchio *et al*, *supra* 1999). US Patent No. 6,270,759 suggests that IL-11 may be  
30 therapeutically useful for a variety of inflammatory conditions including asthma and rhinitis.

[0008] Indicative of the therapeutic interest in IL-11, US Patent Application No. 2007/0190024 describes modified forms of IL-11 with mutations at His 182 (H182) and Asp 186 (D186) which act as agonists and hyperagonists of IL-11.

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[0009] IL-11 exerts its effects *via* association with a specific cell surface receptor (IL-11R $\alpha$ ) as well as the shared receptor subunit gp130. While all IL-6 family cytokines signal through receptor complexes involving one or more gp130 molecules, the IL-11 signaling complex is most similar to that of IL-6 in that it comprises two molecules each of the  
10 cytokine, specific  $\alpha$ -chain receptor and gp130 (Barton *et al*, *J Biol Chem* (2000) 275:36197-36203, 2000).

[0010] While neutralizing antibodies and soluble receptor proteins are a common strategy for inhibiting cytokines, a third class of antagonist molecules are referred to as "cytokine  
15 muteins" which prevent signaling by binding to only one of the two receptor chains. A number of these muteins has previously been described and one, an antagonistic variant of growth hormone, is used clinically to treat acromegaly (Cunningham and Wells, *Science* 244:1081-1085, 1989). Within the IL-6 family of cytokines, cytokine muteins have been described for IL-6, CNTF, LIF and IL-11 (Ehlers *et al*, *J Biol Chem* 270:8158-8163, 1995;  
20 Brakenhoff *et al*, *J Biol Chem* 269:86-93, 1994; Savino *et al*, *Embo J* 13:5863-5870, 1994; Hudson *et al*, *J Biol Chem* 271:11971-11978, 1996; Saggio *et al*, *Embo J* 14; 3045-3054, 1995; Underhill-Day *et al*, *Endocrinology* 144; 3406-3414, 2003). In each case, these cytokine mutein contain specific mutations which prevent binding of cytokine to gp130. In the case of IL-11, a single point mutation, W147A (a tryptophan to alanine substitution at  
25 amino acid residue 147), is sufficient to convert IL-11 from an agonist into an antagonist of IL-11 signaling with the affinity for IL-11R $\alpha$  unchanged (Underhill-Day *et al*, *supra* 2003).

[0011] In addition, structure-function studies have identified various regions of murine and  
30 human IL-11 which are important for IL-11R $\alpha$  binding (Czupryn *et al*, *J. Biol. Chem.* 270 (2): 978-985, 1995; Miyadai *et al*, *Biosci. Biotechnol. Biochem.* 60.3:541-542, 1996;

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Czupryn *et al*, *Ann. N.Y. Acad. Sci.* 762:152-164, 1995; Tacke *et al*, *Eur. J. Biochem.* 265.2:645-655, 1999; Harmegnies *et al*, *Biochem J.* 375(1):23-32, 2003). In particular, residues D165, W166, R169, L172 and L173 at the C-terminal end of the D-helix, and M58, L64 and L67 in the A-B loop were found to contribute to IL-11R $\alpha$  binding.

5

[0012] US Patent Application No. 2007/0190024 describes IL-11 muteins with mutations at His 182 (H182) and Asp 186 (D186) of IL-11 as agonists and hyperagonists of IL-11, but does not suggest antagonists. The IL-11 mutein, W147A IL-11, is an antagonistic variant of IL-11 that prevents the recruitment of gp130 to the IL-11 receptor complex  
10 (Underhill-Day *et al*, *supra* 2003) thereby preventing IL-11 signaling. However, W147A IL-11 has the same affinity for IL-11R $\alpha$  as wild-type IL-11.

[0013] There is a role for IL-11 modulators in therapy. The identification of further IL-11 modulators is required.

15

**SUMMARY**

[0014] Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

[0015] Nucleotide and amino acid sequences are referred to by a sequence identifier number (SEQ ID NO:). The SEQ ID NOs: correspond numerically to the sequence identifiers <400>1 (SEQ ID NO:1), <400>2 (SEQ ID NO:2), etc. A summary of the sequence identifiers is provided in Table 1. A sequence listing is provided after the claims.

[0016] The present invention relates generally to modified forms of mammalian interleukin-11 (referred to hereinafter as "IL-11 muteins") which exhibit enhanced binding to the IL-11 receptor alpha chain (IL-11R $\alpha$ ). More particularly, the IL-11 muteins of the present invention modulate IL-11 signaling and are therefore useful as therapeutics in the treatment or prophylaxis of IL-11-mediated conditions. By "modulate" means up-regulate ("agonize") or down-regulate ("antagonize").

[0017] Accordingly, the present invention provides an IL-11 mutein comprising an amino acid sequence wherein the amino acid sequence AMSAG (using single letter amino acid code) [SEQ ID NO:23] at position 58 to 62 of wild-type mammalian IL-11 is replaced with the amino acid sequence PAIDY (SEQ ID NO:24) or FMQIQ (SEQ ID NO:25). In one embodiment, the IL-11 mutein is in isolated form although the present invention is not to be so limited.

[0018] In another aspect, the IL-11 mutein has, in addition to the mutation at amino acid position 58 to 62 of wild-type mammalian IL-11, a mutation that inhibits its binding to gp130.

30

[0019] In another aspect, the IL-11 mutein has, in addition to the mutation at amino acid

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position 58 to 62 of wild-type mammalian IL-11, a tryptophan at amino acid position 147 of wild-type IL-11 mutated to inhibit its binding to gp130. Reference to "mutated" in this context includes an amino acid substitution, addition and/or deletion.

5 [0020] Specific IL-11 muteins of the present invention include an IL-11 mutein comprising SEQ ID NO:4, amino acids 10 to 178 of SEQ ID NO:4, amino acids 10 to 175 of SEQ ID NO:4, SEQ ID NO:5, amino acids 10 to 178 of SEQ ID NO:5, amino acids 10 to 175 of SEQ ID NO:5, SEQ ID NO:6, amino acids 10 to 178 of SEQ ID NO:6, amino acids 10 to 175 of SEQ ID NO:6, SEQ ID NO:7, amino acids 10 to 178 of SEQ ID NO:7,  
10 amino acids 10 to 175 of SEQ ID NO:7, SEQ ID NO:8, amino acids 10 to 178 of SEQ ID NO:8, amino acids 10 to 175 of SEQ ID NO:8, SEQ ID NO:13, amino acids 10 to 178 of SEQ ID NO:13 or amino acids 10 to 175 of SEQ ID NO:13.

[0021] Other specific IL-11 muteins of the present invention include an IL-11 mutein  
15 comprising SEQ ID NO:9, amino acids 10 to 178 of SEQ ID NO:9, amino acids 10 to 175 of SEQ ID NO:9, SEQ ID NO:10, amino acids 10 to 178 of SEQ ID NO:10, amino acids 10 to 175 of SEQ ID NO:10, SEQ ID NO:11, amino acids 10 to 178 of SEQ ID NO:11, amino acids 10 to 175 of SEQ ID NO:11, SEQ ID NO:12, amino acids 10 to 178 of SEQ ID NO:12, amino acids 10 to 175 of SEQ ID NO:12, SEQ ID NO:14, amino acids 10 to  
20 178 of SEQ ID NO:14, amino acids 10 to 175 of SEQ ID NO:14, SEQ ID NO:15, amino acids 10 to 178 of SEQ ID NO:15, amino acids 10 to 175 of SEQ ID NO:15, SEQ ID NO:16, amino acids 10 to 178 of SEQ ID NO:16, amino acids 10 to 175 of SEQ ID NO:16, SEQ ID NO:17, amino acids 10 to 178 of SEQ ID NO:17, amino acids 10 to 175 of SEQ ID NO:17, SEQ ID NO:18, amino acids 10 to 178 of SEQ ID NO:18, amino acids  
25 10 to 175 of SEQ ID NO:18, SEQ ID NO:19, amino acids 10 to 178 of SEQ ID NO:19, amino acids 10 to 175 of SEQ ID NO:19, SEQ ID NO:20, amino acids 10 to 178 of SEQ ID NO:20, amino acids 10 to 175 of SEQ ID NO:20, SEQ ID NO:21, amino acids 10 to 178 of SEQ ID NO:21 or amino acids 10 to 175 of SEQ ID NO:21.

30 [0022] The present invention also provides a nucleic acid sequence encoding an IL-11 mutein described herein.

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[0023] In another aspect the present invention provides an IL-11 mutein which is PEGylated.

- 5 [0024] In another aspect the present invention contemplates a method for the treatment of an IL-11-mediated condition, the method comprising administering to said subject an effective amount of an IL-11 mutein of the present invention.

[0025] The present invention is further directed to the use of an IL-11 mutein of the present invention in the manufacture of a medicament for the treatment of an  
10 IL-11-mediated condition.

[0026] An IL-11-mediated condition includes (a) any condition which benefits or might benefit from increasing treatment with exogenous IL-11 or an IL-11 agonist, for example  
15 thrombocytopenia, rheumatoid arthritis, inflammatory bowel disease, infertility, and mucosal damage from chemotherapy and/or radiation therapy; and (b) any condition which benefits or might benefit from treatment with an IL-11 antagonist to reduce or block the activity of endogenous IL-11, for example conditions that result in diminished total bone mass, including metastatic bone cancer, myeloma, Paget's disease of the bone and  
20 osteoporosis, and fertility (i.e. an IL-11 antagonist may be used for contraception). In one embodiment, the IL-11 mutein antagonist comprises a substitution of AMSAG at amino acid position 58 to 62 together with a mutation that disrupts binding to gp130. An example of the latter is a mutation at amino acid 147 (e.g. a W147 mutation or a W147A or W147C substitution).

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[0027] The present invention extends to compositions comprising IL-11 muteins of the present invention and one or more pharmaceutically acceptable carriers and/or diluents and/or excipient.



**TABLE 1**  
*Summary of sequence identifiers*

SEQUENCE ID NO:	DESCRIPTION
1	Human mature IL-11 amino acid sequence
2	Murine mature IL-11 amino acid sequence
3	Macaque Mature IL-11 amino acid sequence
4	Macaque PAIDY IL-11 mutein amino acid sequence
5	Human PAIDY IL-11 mutein amino acid sequence
6	Murine PAIDY IL-11 mutein amino acid sequence
7	Human FMQIQ IL-11 mutein amino acid sequence
8	Murine FMQIQ IL-11 mutein amino acid sequence
9	Human PAIDY and W147A IL-11 mutein amino acid sequence
10	Murine PAIDY and W147A IL-11 mutein amino acid sequence
11	Human PAIDY and W147C IL-11 mutein amino acid sequence
12	Murine PAIDY and W147C IL-11 mutein amino acid sequence
13	Macaque FMQIQ IL-11 mutein amino acid sequence
14	Macaque PAIDY and W147A IL-11 mutein amino acid sequence
15	Macaque PAIDY and W147C IL-11 mutein amino acid sequence
16	Human FMQIQ and W147A IL-11 mutein amino acid sequence
17	Murine FMQIQ and W147A IL-11 mutein amino acid sequence
18	Macaque FMQIQ and W147A IL-11 mutein amino acid sequence
19	Human FMQIQ and W147C IL-11 mutein amino acid sequence
20	Murine FMQIQ and W147C IL-11 mutein amino acid sequence
21	Macaque FMQIQ and W147C IL-11 mutein amino acid sequence
22	N-terminally tagged Human PAIDY and W147C IL-11 mutein amino acid sequence
23	Amino acids at position 58 to 62 of wild-type IL-11
24	Substitution amino acids at position 58 to 62 of wild-type IL-11
25	Substitution amino acids at position 58 to 62 of wild-type IL-11

[0028] A summary of amino acid single and three letter codes is provided in Table 2.

**TABLE 2**  
*Amino Acid Abbreviations*

5

<b>Amino Acid</b>	<b>Three-letter Abbreviation</b>	<b>One-letter Symbol</b>
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

## **BRIEF DESCRIPTION OF THE FIGURES**

[0029] **Figures 1a to 1g** disclose the amino acid sequences of human, murine and monkey IL-11 muteins of the present invention.

## DETAILED DESCRIPTION

- 5 [0030] As used herein, the singular forms "a", "an" and "the" include plural aspects unless the context clearly dictates otherwise. Thus, for example, reference to "a mutein" includes a single mutein, as well as two or more muteins; reference to "an agent" includes a single agent, as well as two or more agent; reference to "the invention" includes single and multiple aspects of the invention; and so forth.
- 10 [0031] The term "IL-11" or its full name "interleukin-11" as used herein includes all mature forms of wild-type mammalian IL-11, including murine, macaque and human, and all truncated forms of such IL-11 that retain IL-11 activity, i.e. the ability to bind with IL-11R $\alpha$  and form a functional receptor complex with gp130. Mature human IL-11 (SEQ ID NO:1) is a 178 amino acid protein (i.e. lacking the 21 amino acid leader sequence of  
15 NP\_000632, NCBI protein database Accession Number), mature murine IL-11 (SEQ ID NO:2) is a 178 amino acid protein (i.e. lacking the 21 amino acid leader sequence of NP\_032376, NCBI protein database Accession Number) and mature macaque IL-11 (SEQ ID NO:3) is a 178 amino acid protein (i.e. lacking the 21 amino acid leader sequence of P20808, NCBI protein database Accession Number).
- 20 [0032] The term "IL-11 mutein" as used herein refers to an IL-11 in which the amino acid sequence of the wild-type protein has been altered by amino acid substitutions, additions and/or deletions to provide enhanced binding to the IL-11R $\alpha$  chain to generate an IL-11 mutein agonist or, in the case of an IL-11 mutein antagonist, the amino acid sequence has  
25 been further altered by amino acid substitutions, additions and/or deletions to antagonize IL-11 signaling by inhibiting the formation of an IL-11 receptor complex with gp130 while retaining enhanced binding to the IL-11R $\alpha$  chain. Particularly, the IL-11 mutein is based on a human, macaque or murine IL-11, and more particularly human IL-11. The IL-11 muteins may be further modified, for example to increase their *in vivo* half life, including  
30 for example by the attachment of other elements such as a PEG group. Methods for the

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PEGylation of peptides are well known in the art. IL-11 muteins may sometimes be referred to as IL-11 mutant proteins or as IL-11 mutants.

[0033] The expression "enhanced binding to the IL-11 receptor alpha (IL-11R $\alpha$ ) chain" when used in relation to the IL-11 muteins of the present invention means that the IL-11 mutein exhibits a greater affinity for the IL-11R $\alpha$  chain than does the corresponding wild-type IL-11 as determined by competition ELISA.

[0034] The terms "antagonist", "agonist" and "compound" may each be used herein to refer to the IL-11 muteins described throughout the specification. The terms also encompass pharmaceutically acceptable and pharmacologically active forms thereof, including salts.

[0035] The term "effective amount" as used herein means a sufficient amount of an IL-11 mutein to provide the desired physiological and/or therapeutic effect such as to antagonize IL-11 signaling. In addition, the effect may be an amelioration of the symptoms of an IL-11-mediated condition. Undesirable effects, e.g. side effects, are sometimes manifested along with the desired physiological and/or therapeutic effect; hence, a practitioner balances the potential benefits against the potential risks when determining what is an appropriate "effective amount". The exact amount required will vary from subject to subject, depending on the species, age and general condition of the subject, mode of administration and the like. Thus, it may not be possible to specify an exact "effective amount". However, an appropriate "effective amount" in any individual case may be determined by one of ordinary skill in the art using routine experimentation. One of ordinary skill in the art would be able to determine the required amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

[0036] Insofar as one embodiment of the present invention relates to the use of an IL-11 mutein, the effective amount includes from about 10 $\mu$ g/kg body weight to 20mg/kg body weight of antibody such as 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 $\mu$ g/kg body weight, 100,

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200, 300, 400, 500, 600, 700, 800, 900, 1000µg/kg body weight or 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20mg/kg body weight. Similar amounts are provided for single or combination therapy.

5 [0037] Reference to "a condition mediated by IL-11" or an "IL-11-mediated condition" includes (a) any condition which benefits or might benefit from increasing treatment with exogenous IL-11 or an IL-11 agonist, for example thrombocytopenia, rheumatoid arthritis, inflammatory bowel disease, infertility, and mucosal damage from chemotherapy and/or radiation therapy; and (b) any condition which benefits or might benefit from treatment  
10 with an IL-11 antagonist to reduce or block the activity of endogenous IL-11, for example conditions that result in diminished total bone mass, including metastatic bone cancer, myeloma, Paget's disease of the bone and osteoporosis, and fertility (i.e. an IL-11 antagonist may be used for contraception).

15 [0038] A "pharmaceutically acceptable" carrier and/or diluent is a pharmaceutical vehicle comprised of a material that is not biologically or otherwise undesirable, i.e. the material may be administered to a subject along with the selected mutein without causing any or a substantial adverse reaction. Carriers and diluents may include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, agents used for adjusting  
20 tonicity, buffers, chelating agents, and absorption delaying agents and the like.

[0039] Similarly, a "pharmacologically acceptable" salt of a compound as provided herein is a salt that is not biologically or otherwise undesirable.

25 [0040] The terms "treating" and "treatment" as used herein refer to therapeutic treatment. For example, treatment may result in a reduction in severity and/or the frequency of symptoms of the condition, the elimination of symptoms and/or underlying cause of the condition, the prevention of the occurrence of symptoms of the condition and/or their underlying cause and improvement or remediation or amelioration of damage. Hence, the  
30 treatment may not result in a "cure" but rather an amelioration of symptoms. In addition, treatment may not commence until an exacerbated event occurs. In this context, the term

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"prophylaxis" also applies to the prevention or treatment of a likelihood of an event associated with the condition occurring.

[0041] The terms "treating" and "treatment" as used herein also refer to the reduction of  
5 one or more symptoms or characteristics associated with the conditions.

[0042] A "subject" as used herein refers to an animal, preferably a mammal and more preferably a human who can benefit from the pharmaceutical compositions and methods of the present invention. Other preferred mammals are laboratory test animals, examples of  
10 which include mice, rats, rabbits, guinea pigs, hamsters, cats and dogs. There is no limitation on the type of animal that could benefit from the presently described pharmaceutical compositions and methods. A subject regardless of whether a human or non-human animal may be referred to as an individual, patient, animal or recipient as well as subject. The methods of the present invention have applications in human medicine and  
15 veterinary medicine.

[0043] It is shown herein that IL-11 muteins with enhanced binding to the IL-11R $\alpha$  chain are produced when the amino acid sequence AMSAG (using single letter amino acid code) [SEQ ID NO:23] at positions 58 to 62 of wild-type mammalian IL-11 is replaced with the  
20 amino acid sequence PAIDY (SEQ ID NO:24) or FMQIQ (SEQ ID NO:25).

[0044] Accordingly, the present invention provides an IL-11 mutein wherein the amino acid sequence AMSAG (SEQ ID NO:23) at positions 58 to 62 of wild-type mammalian IL-11 is replaced with the amino acid sequence PAIDY (SEQ ID NO:24) or FMQIQ (SEQ ID  
25 NO:25).

[0045] One aspect of present invention provides an IL-11 mutein wherein the amino acid sequence AMSAG at positions 58 to 62 of wild-type human IL-11 (SEQ ID NO:1), murine IL-11 (SEQ ID NO:2) or macaque IL-11 (SEQ ID NO:3) is replaced with the amino acid  
30 sequence PAIDY (SEQ ID NO:24).

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[0046] Another aspect of the present invention provides an IL-11 mutein wherein the amino acid sequence AMSAG at positions 58 to 62 of wild-type human IL-11 (SEQ ID NO:1), murine IL-11 (SEQ ID NO:2) or macaque IL-11 (SEQ ID NO:3) is replaced with the amino acid sequence FMQIQ (SEQ ID NO:25).

5

[0047] In one aspect, an IL-11 mutein of the present invention has a 10-fold, more particularly a 15-fold, and even more particularly a 20-fold higher binding affinity for the IL-11R $\alpha$  chain than the binding affinity of the corresponding wild-type IL-11.

10 [0048] The inclusion of additional mutations that retain enhanced binding to the IL-11R $\alpha$  chain but inhibit binding to gp130 provides IL-11 mutein antagonists which compete with IL-11 for binding to the IL-11R $\alpha$  chain but do not form an IL-11 receptor complex with gp130.

15 [0049] Accordingly, in another aspect the IL-11 mutein may have, in addition to the mutations at amino acid positions 58 to 62 of wild-type mammalian IL-11, an additional mutation that inhibits binding to gp130.

[0050] A mutation of the tryptophan (W) residue at amino acid position 147 of wild-type murine IL-11 to alanine (A) is known to inhibit binding of the resulting IL-11 mutant to gp130. It is shown herein that a substitution mutation of the tryptophan residue at amino acid position 147 of wild-type murine IL-11 to cysteine inhibits binding of the resulting IL-11 mutant to gp130. Reference to "mutation" includes an amino acid substitution, addition and/or deletion. A substitution mutation is conveniently described herein as  
25 W147A or W147C to denote a change from a tryptophan (W) to an alanine (A) or cysteine (C).

[0051] Accordingly, in another aspect the IL-11 mutein may, in addition to the mutation at amino acid positions 58 to 62 of wild-type mammalian IL-11, have the tryptophan at  
30 amino acid position 147 of wild-type IL-11 mutated to inhibit its binding to gp130.



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[0052] In another aspect, the tryptophan at amino acid position 147 of wild-type mammalian IL-11 is mutated to an alanine or a cysteine.

[0053] In another aspect, the IL-11 mutein may, in addition to the mutation at amino acid positions 58 to 62 of wild-type human IL-11 (SEQ ID NO:1), murine IL-11 (SEQ ID NO:2) or macaque IL-11 (SEQ ID NO:3) have an additional mutation which is inhibit its binding to gp130.

[0054] In another aspect, the IL-11 mutein may, in addition to the mutation at amino acid positions 58 to 62 of wild-type human IL-11 (SEQ ID NO:1), murine IL-11 (SEQ ID NO:2) or macaque IL-11 (SEQ ID NO:3) have the tryptophan residue at amino acid position 147 of wild-type mammalian IL-11 mutated to inhibit binding to gp130. In another aspect the tryptophan residue at amino acid position 147 of wild-type mammalian IL-11 is mutated to an alanine or a cysteine.

15

[0055] The present inventors have observed that up to nine N-terminal amino acid residues can be removed from murine IL-11 without loss of activity. Wang *et al*, *Eur J Biochem.* 269(1):61-68, 2002, removed ten N-terminal amino acid residues from human IL-11. Barton *et al*, *J Biol Chem.* 274(9):5755-61, 1999, report that amino acid residue thirteen of murine IL-11 forms part of the site II gp130 binding site and that substitution causes a loss in activity, suggesting that up to the first twelve N-terminal amino acid residues may be removed without significant loss of activity. US Patent Application No. 20070190024 which describes IL-11 muteins that are IL-11 agonists suggests that the first thirteen N-terminal amino acid residues may be removed without loss of activity.

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[0056] It has also been reported that up to the last three, but not four, amino acid residues may be removed from the C-terminus of human IL-11 without a loss of activity (Czupryn *et al*, *supra* 1995).

[0057] In another aspect the IL-11 muteins of the present invention may, in addition to the mutations at amino acid positions 58 to 62 of wild-type IL-11, have up to the first thirteen,

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preferably only up to the first twelve, N-terminal amino acid residues of the corresponding wild-type IL-11 deleted and/or have up to the last three C-terminal amino acid residues of the corresponding wild-type IL-11 deleted.

5 [0058] Specific IL-11 muteins of the present invention include an IL-11 mutein comprising SEQ ID NO:4, amino acids 10 to 178 of SEQ ID NO:4, amino acids 10 to 175 of SEQ ID NO:4, SEQ ID NO:5, amino acids 10 to 178 of SEQ ID NO:5, amino acids 10 to 175 of SEQ ID NO:5, SEQ ID NO:6, amino acids 10 to 178 of SEQ ID NO:6, amino acids 10 to 175 of SEQ ID NO:6, SEQ ID NO:7, amino acids 10 to 178 of SEQ ID NO:7,  
10 amino acids 10 to 175 of SEQ ID NO:7, SEQ ID NO:8, amino acids 10 to 178 of SEQ ID NO:8, amino acids 10 to 175 of SEQ ID NO:8, SEQ ID NO:13, amino acids 10 to 178 of SEQ ID NO:13 or amino acids 10 to 175 of SEQ ID NO:13.

[0059] Other specific IL-11 muteins of the invention include an IL-11 mutein comprising  
15 SEQ ID NO:9, amino acids 10 to 178 of SEQ ID NO:9, amino acids 10 to 175 of SEQ ID NO:9, SEQ ID NO:10, amino acids 10 to 178 of SEQ ID NO:10, amino acids 10 to 175 of SEQ ID NO:10, SEQ ID NO:11, amino acids 10 to 178 of SEQ ID NO:11, amino acids 10 to 175 of SEQ ID NO:11, SEQ ID NO:12, amino acids 10 to 178 of SEQ ID NO:12, amino acids 10 to 175 of SEQ ID NO:12, SEQ ID NO:14, amino acids 10 to 178 of SEQ ID  
20 NO:14, amino acids 10 to 175 of SEQ ID NO:14, SEQ ID NO:15, amino acids 10 to 178 of SEQ ID NO:15, amino acids 10 to 175 of SEQ ID NO:15, SEQ ID NO:16, amino acids 10 to 178 of SEQ ID NO:16, amino acids 10 to 175 of SEQ ID NO:16, SEQ ID NO:17, amino acids 10 to 178 of SEQ ID NO:17, amino acids 10 to 175 of SEQ ID NO:17, SEQ ID NO:18, amino acids 10 to 178 of SEQ ID NO:18, amino acids 10 to 175 of SEQ ID  
25 NO:18, SEQ ID NO:19, amino acids 10 to 178 of SEQ ID NO:19, amino acids 10 to 175 of SEQ ID NO:19, SEQ ID NO:20, amino acids 10 to 178 of SEQ ID NO:20, amino acids 10 to 175 of SEQ ID NO:20, SEQ ID NO:21, amino acids 10 to 178 of SEQ ID NO:21 or amino acids 10 to 175 of SEQ ID NO:21.

30 [0060] IL-11 from monkey (SEQ ID NO:3) and mouse (SEQ ID NO:2) demonstrate considerable identity with the human sequence (SEQ ID NO:1, ~94% and ~87% identity

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respectively). All these proteins have full cross-species reactivity indicating that their tertiary structures are similar and other functionally critical residues are probably conserved. (Czupryn *et al, supra* 1995). In view of the cross reactivity of IL-11 from different species it is clear that IL-11 can tolerate some level of amino acid variation and retain IL-11 activity. Accordingly, the IL-11 muteins of the present invention include IL-11 muteins comprising sequences that are at least 85% identical, preferably at least 90% identical and more preferably at least 94% identical to any one of those of SEQ ID NO:4, amino acids 10 to 178 of SEQ ID NO:4, amino acids 10 to 175 of SEQ ID NO:4, SEQ ID NO:5, amino acids 10 to 178 of SEQ ID NO:5, amino acids 10 to 175 of SEQ ID NO:5, SEQ ID NO:6, amino acids 10 to 178 of SEQ ID NO:6, amino acids 10 to 175 of SEQ ID NO:6, SEQ ID NO:7, amino acids 10 to 178 of SEQ ID NO:7, amino acids 10 to 175 of SEQ ID NO:7, SEQ ID NO:8, amino acids 10 to 178 of SEQ ID NO:8, amino acids 10 to 175 of SEQ ID NO:8, SEQ ID NO:9, amino acids 10 to 178 of SEQ ID NO:9, amino acids 10 to 175 of SEQ ID NO:9, SEQ ID NO:10, amino acids 10 to 178 of SEQ ID NO:10, amino acids 10 to 175 of SEQ ID NO:10, SEQ ID NO:11, amino acids 10 to 178 of SEQ ID NO:11, amino acids 10 to 175 of SEQ ID NO:11, SEQ ID NO:12, amino acids 10 to 178 of SEQ ID NO:12, amino acids 10 to 175 of SEQ ID NO:12, SEQ ID NO:13, amino acids 10 to 178 of SEQ ID NO:13, amino acids 10 to 175 of SEQ ID NO:13, SEQ ID NO:14, amino acids 10 to 178 of SEQ ID NO:14, amino acids 10 to 175 of SEQ ID NO:14, SEQ ID NO:15, amino acids 10 to 178 of SEQ ID NO:15, amino acids 10 to 175 of SEQ ID NO:15, SEQ ID NO:16, amino acids 10 to 178 of SEQ ID NO:16, amino acids 10 to 175 of SEQ ID NO:16, SEQ ID NO:17, amino acids 10 to 178 of SEQ ID NO:17, amino acids 10 to 175 of SEQ ID NO:17, SEQ ID NO:18, amino acids 10 to 178 of SEQ ID NO:18, amino acids 10 to 175 of SEQ ID NO:18, SEQ ID NO:19, amino acids 10 to 178 of SEQ ID NO:19, amino acids 10 to 175 of SEQ ID NO:19, SEQ ID NO:20, amino acids 10 to 178 of SEQ ID NO:20, amino acids 10 to 175 of SEQ ID NO:20, SEQ ID NO:21, amino acids 10 to 178 of SEQ ID NO:21 or amino acids 10 to 175 of SEQ ID NO:21.

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[0061] The present invention also provides nucleic acid sequences encoding the IL-11 muteins described herein. A nucleic acid sequence or nucleic acid includes a polynucleotide or nucleic acid molecule.

5 [0062] The present invention employs conventional molecular biology, microbiology, and recombinant DNA techniques to modify wild-type IL-11 nucleic acid sequences to produce the IL-11 muteins of the present invention. The techniques are well known in the art and are described in various publications, such as Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press,  
10 Cold Spring Harbor, N.Y, 1989.; *DNA Cloning: A Practical Approach*, Volumes I and II (D. N. Glover ed. 1985), Ausubel, *et al.* (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., 1994, Sidhu *et al*, *Methods Enzymol* 328:333-363, 2000) and Kunkel *et al*, *Methods Enzymol* 204: 1991.

15 [0063] The terms "polynucleotide", "nucleic acid" or "nucleic acid molecule" refer to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules"), or any phosphoester analogs thereof, such as phosphorothioates and thioesters, in single stranded form, double-stranded  
20 form or otherwise.

[0064] The terms "polynucleotide sequence", "nucleic acid sequence" or "nucleotide sequence" refer to a series of nucleotide bases (also referred to as "nucleotides") in a nucleic acid, such as DNA or RNA, and means any chain of two or more nucleotides.

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[0065] The terms "coding sequence" or a sequence "encoding" an expression product, such as a RNA, polypeptide, protein, or enzyme, is a nucleotide sequence that, when expressed, results in the production of the product.

30 [0066] The term "gene" means a DNA sequence that codes for or corresponds to a particular sequence of ribonucleotides or amino acids which comprise all or part of one or

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more RNA molecules, proteins or enzymes, and may or may not include regulatory DNA sequences, such as promoter sequences, which determine, for example, the conditions under which the gene is expressed. Genes may be transcribed from DNA to RNA which may or may not be translated into an amino acid sequence.

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[0067] The term "amplification" of nucleotide sequence as used herein may denote the use of the polymerase chain reaction (PCR) to increase the concentration of a particular nucleotide sequence within a mixture of nucleotide sequence sequences. Saiki, *et al*, *Science* 239:487, 1988 provide a description of PCR.

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[0068] The term "oligonucleotide" refers to a nucleic acid, generally of at least 10, particularly at least 15, and more particularly at least 20 nucleotides, particularly no more than 100 nucleotides that may be hybridizable to a genomic DNA molecule, a cDNA molecule, or an mRNA molecule encoding a gene, mRNA, cDNA, or other nucleic acid of interest. Oligonucleotides can be labeled for example, by incorporation of  $^{32}\text{P}$ -nucleotides,  $^3\text{H}$ -nucleotides,  $^{14}\text{C}$ -nucleotides,  $^{35}\text{S}$ -nucleotides or nucleotides to which a label, such as biotin, has been covalently conjugated. In one embodiment, a labeled oligonucleotide can be used as a probe to detect the presence of a nucleic acid. In another embodiment, oligonucleotides (one or both of which may be labeled) can be used as PCR primers, either for cloning full length or a fragment of the gene, or to detect the presence of nucleic acids. Generally, oligonucleotides are prepared synthetically, preferably on a nucleic acid synthesizer.

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[0069] The sequence of any nucleic acid (for example, a nucleic acid encoding a wild-type IL-11 protein or an IL-11 mutein) may be sequenced by any method known in the art such as by chemical sequencing or enzymatic sequencing. "Chemical sequencing" of DNA may be done by the method of Maxam and Gilbert (*Proc. Natl. Acad. Sci. USA* 74(2): 560-564, 1977), in which DNA is randomly cleaved using individual base-specific reactions. "Enzymatic sequencing" of DNA may be done by the method of Sanger (Sanger *et al*, *Proc. Natl. Acad. Sci. USA* 74(12):5463 5467, 1977).

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[0070] Nucleic acids of the present invention may be flanked by natural regulatory (expression control) sequences, or may be associated with heterologous sequences, including promoters, internal ribosome entry sites (IRES) and other ribosome binding site sequences, enhancers, response elements, suppressors, signal sequences, polyadenylation  
5 sequences, introns, 5'-and 3'-non-coding regions, and the like.

[0071] A "promoter" or "promoter sequence" is a DNA regulatory region capable of binding an RNA polymerase in a cell and initiating transcription of a coding sequence. A promoter sequence is generally bounded at its 3' terminus by the transcription initiation site  
10 and extends upstream in the 5' direction to include the minimum number of bases or elements necessary to initiate transcription at any level. A transcription initiation site as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase may be found within the promoter sequence. The promoter may be operably associated with other expression control sequences, including enhancer and repressor  
15 sequences or with a nucleic acid of the invention. Promoters which may be used to control gene expression include, but are not limited to, the cytomegalovirus (CMV) promoter (US Patent Nos. 5,385,839 and 5,168,062) and the SV40 early promoter region (Benoist, *et al*, *Nature* 290:304-310, 1981).

[0072] A coding sequence is "under the control of", "functionally associated with" or "operably associated with" transcriptional and translational control sequences in a cell when the sequences direct RNA polymerase mediated transcription of the coding sequence into RNA, preferably mRNA, which then may be trans-RNA spliced (if it contains introns) and, optionally, translated into a protein encoded by the coding sequence.  
20

[0073] The terms "express" and "expression" mean allowing or causing the information in a gene, RNA or DNA sequence to be converted into a product; for example, producing a protein by activating the cellular functions involved in transcription and translation of a nucleotide sequence. A DNA sequence is expressed in or by a cell to form an "expression  
30 product" such as RNA (such as mRNA) or a protein (such as an IL-11 mutein). The expression product itself may also be said to be "expressed" by the cell.

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[0074] The terms "vector", "cloning vector" and "expression vector" mean the vehicle (such as a plasmid) by which a DNA or RNA sequence can be introduced into a host cell, so as to transform the host and, optionally, promote expression and/or replication of the  
5 introduced sequence.

[0075] The term "transfection" or "transformation" means the introduction of a nucleic acid into a cell. These terms may refer to the introduction of a nucleic acid encoding an IL-11 mutein into a cell. The introduced gene or sequence may be called a "clone". A host cell  
10 that receives the introduced DNA or RNA has been "transformed" and is a "transformant" or a "clone". The DNA or RNA introduced to a host cell can come from any source, including cells of the same genus or species as the host cell, or cells of a different genus or species.

[0076] The term "host cell" means any cell of any organism that is selected, modified, transfected, transformed, grown, or used or manipulated in any way, for the production of a substance by the cell, for example the expression or replication, by the cell, of a gene, a  
15 DNA or RNA sequence, a protein or an enzyme.

[0077] The term "expression system" means a host cell and compatible vector which, under suitable conditions, can express a protein or nucleic acid which is carried by the vector and introduced to the host cell. Common expression systems include *E.coli* host  
20 cells and plasmid vectors, insect host cells and Baculovirus vectors, and mammalian host cells and vectors.

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[0078] The present invention contemplates any slight modifications of the amino acid or nucleotide sequences which corresponds to or encodes the IL-11 muteins of the sequences described herein excluding modifications that would change the amino acid segment corresponding to amino acids 58 to 62 of wild-type mammalian IL-11 which segment will  
30 have the sequence PAIDY (SEQ ID NO:24) or FMQIQ (SEQ ID NO:25). In particular, the present invention contemplates sequence conservative variants of the nucleic acids which

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encode the IL-11 muteins of the invention. "Sequence-conservative variants" of a polynucleotide sequence are those in which a change of one or more nucleotides in a given codon results in no alteration in the amino acid encoded at that position. Function-conservative variants of the IL-11 muteins of the invention are also contemplated by the present invention. "Function-conservative variants" are those in which one or more amino acid residues in a protein have been changed without altering the overall conformation and function of the protein, including, but, by no means, limited to, replacement of an amino acid with one having similar properties. Amino acids with similar properties are well known in the art. For example, polar/hydrophilic amino acids which may be interchangeable include asparagine, glutamine, serine, cysteine, threonine, lysine, arginine, histidine, aspartic acid and glutamic acid; nonpolar/hydrophobic amino acids which may be interchangeable include glycine, alanine, valine, leucine, isoleucine, proline, tyrosine, phenylalanine, tryptophan and methionine; acidic amino acids which may be interchangeable include aspartic acid and glutamic acid and basic amino acids which may be interchangeable include histidine, lysine and arginine. Preferably, function-conservative variants of the IL-11 muteins of the invention have less than 20, more preferably less than 15, more preferably less than 10 amino acid changes.

[0079] Also included in the present invention are IL-11 muteins wherein the amino acid sequence AMSAG (SEQ ID NO:23) at positions 58 to 62 of wild-type mammalian IL-11 is replaced with the amino acid sequence PAIDY (SEQ ID NO:24) or FMQIQ (SEQ ID NO:25) and comprising amino acid sequences which are at least 85% identical, particularly at least 90% identical, more particularly at least 94% identical (e.g. 94%, 95%, 96%, 97%, 98%, 99%) to the amino acid sequences described herein when the comparison is performed by a BLAST algorithm wherein the parameters of the algorithm are selected to give the largest match between the respective sequences over the entire length of the respective reference sequences.

[0080] Sequence identity refers to exact matches between the amino acids of two sequences which are being compared.



[0081] Descriptions for BLAST algorithms can be found in the following references which herein incorporated by reference: BLAST ALGORITHMS: Altschul *et al*, *J. Mol. Biol.* 215:403-410, 1990; Altschul *et al*, *Nucleic Acids Res.* 25:3389-3402, 1997; Altschul, *J. Mol. Biol.* 219:555-565, 1991.

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[0082] The IL-11 muteins of the present invention may be produced recombinantly, for example, in an *E.coli* expression system. Transformation can be by any known method for introducing polynucleotides into a host cell. Methods for introduction of heterologous polynucleotides into mammalian cells are well known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene-mediated transfection, 10 protoplast fusion, electroporation and encapsulation of the polynucleotide(s) in liposomes, biolistic injection and direct microinjection of the DNA into nuclei. In addition, nucleic acid molecules may be introduced into mammalian cells by viral vectors. Methods of transforming cells are well known in the art. See, for example, US Patent Nos. 4,399,216; 15 4,912,040; 4,740,461 and 4,959,455.

[0083] In one aspect, the present invention provides a method for the production of an IL-11 mutein of the invention, said method comprising cloning a nucleic acid sequence encoding an IL-11 mutein into an appropriate vector, transforming a host cell line with the 20 vector, and culturing the transformed host cell line under conditions suitable for the expression of the antibodies of the present invention.

[0084] Vectors available for cloning and expression in host cell lines are well known in the art, and include but are not limited to vectors for cloning and expression in mammalian cell 25 lines, vectors for cloning and expression in bacterial cell lines and vectors for cloning and expression insect cell lines. The IL-11 muteins can be recovered using standard protein purification methods.

[0085] In another aspect, the present invention provides nucleic acid sequences encoding 30 IL-11 muteins having the amino acid sequences shown in SEQ ID NOs:5 to 12.

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[0086] In still a further aspect, the present invention provides host cell lines transformed with the vectors of the present invention. Host cell lines include, but are not limited to, bacterial cells, such as *E. coli* and mammalian cell lines.

5 [0087] Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC). These include, inter alia, Chinese hamster ovary (CHO) cells, NSO, SP2 cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), A549 cells, 3T3 cells, and a number  
10 of other cell lines. Mammalian host cells include human, mouse, rat, dog, monkey, pig, goat, bovine, horse and hamster cells. Cell lines of particular preference are selected through determining which cell lines have high expression levels. Other cell lines that may be used are insect cell lines, such as Sf9 cells, amphibian cells, bacterial cells, plant cells and fungal cells. When recombinant expression vectors encoding the heavy chain or  
15 antigen-binding portion thereof, the light chain and/or antigen-binding portion thereof are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown.

20

[0088] The IL-11 muteins can be recovered from the culture medium using standard protein purification methods. Further, expression of IL-11 muteins of the invention from production cell lines can be enhanced using a number of known techniques. For example, the glutamine synthetase gene expression system (the GS system) is a common approach  
25 for enhancing expression under certain conditions. The GS system is discussed in whole or part in connection with European Patent Nos. 0 216 846, 0 256 055, and 0 323 997 and European Patent Application No. 89303964.4.

[0089] It is likely that the IL-11 muteins expressed by different cell lines or in transgenic  
30 animals will have different glycosylation from each other. However, all IL-11 muteins encoded by the nucleic acid molecules provided herein, or comprising the amino acid

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sequences provided herein are part of the invention, regardless of the glycosylation of the IL-11 muteins.

5 [0090] In a further aspect, the present invention provides a human or murine IL-11 muteins that have been further modified to enhance their pharmacokinetic properties and half life *in vivo*. Modifications include PEGylation with polyethylene glycol, (Clark *et al*, *J Biol Chem.* 271(36):21969-77, 1996), fusions to large long lived proteins such as albumin (Yeh *et al*, *Proc Natl Acad Sci U S A.* 89(5):1904-8, 1992) or the Fc portion of an Ig (Ashkenazi and Chamow, *Curr Opin Immunol.* 9(2):195-200, 1997) and the  
10 introduction of glycosylation sites (Keyt *et al*, *Proc Natl Acad Sci USA.* 91(9):3670-4, 1994).

[0091] An aspect of the invention provides IL-11 mutein antagonists that are PEGylated.

15 [0092] One aspect of the present invention provides IL-11 muteins having the amino acid sequences of SEQ ID NO:11, amino acids 10 to 178 of SEQ ID NO:11, amino acids 10 to 175 of SEQ ID NO:11, SEQ ID NO:12, amino acids 10 to 178 of SEQ ID NO:12 amino acids 10 to 175 of SEQ ID NO:12, SEQ ID NO:15, amino acids 10 to 178 of SEQ ID NO:15, amino acids 10 to 175 of SEQ ID NO:15, SEQ ID NO:19, amino acids 10 to 178  
20 of SEQ ID NO:19, amino acids 10 to 175 of SEQ ID NO:19, SEQ ID NO:20, amino acids 10 to 178 of SEQ ID NO:20, amino acids 10 to 175 of SEQ ID NO:20, SEQ ID NO:21, amino acids 10 to 178 of SEQ ID NO:21 or amino acids 10 to 175 of SEQ ID NO:21 that are PEGylated.

25 [0093] The IL-11 muteins of the invention may be conveniently supplied in compositions suitable for pharmaceutical use. Such compositions are another aspect of the present invention.

[0094] Administration may be systemic or local. Systemic administration is particularly  
30 useful. Reference to "systemic administration" includes intra-articular, intravenous,

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intraperitoneal, and subcutaneous injection, infusion, as well as administration *via* oral, rectal and nasal routes, or *via* inhalation.

[0095] Compositions suitable for systemic use include sterile aqueous solutions (where  
5 water soluble), sterile powders for the extemporaneous preparation of sterile injectable  
solutions, and sterile powders for inhalation. It must be stable under the conditions of  
manufacture and storage and must be preserved against the contaminating action of  
microorganisms such as bacteria and fungi. The carrier can be any pharmaceutically  
10 acceptable carriers and/or diluent, for example, water, ethanol, polyol (for example,  
glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures  
thereof and vegetable oils. The proper fluidity can be maintained, for example, by the use  
of surfactants. Various anti-bacterial and anti-fungal agents, for example, parabens,  
chlorobutanol, phenol, sorbic acid, thimerosal and the like may be included. In many  
15 cases, it will be preferable to include agents to adjust tonicity, for example, sugars or  
sodium chloride. Prolonged absorption of the injectable compositions can be brought about  
by the use in the compositions of agents delaying absorption, for example, aluminum  
monostearate and gelatin.

[0096] Sterile solutions are prepared by incorporating the active in the required amount in  
20 the appropriate solvent and optionally with other active ingredients and excipients as  
required, followed by filtered sterilization or other appropriate means of sterilization. In  
the case of sterile powders, suitable methods of preparation include vacuum drying and the  
freeze-drying technique which yield a powder of active ingredient plus any additionally  
desired ingredient which can be made at an appropriate particle size.

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[0097] When the active is suitably protected, it may be orally administered, for example,  
with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or  
soft shell gelatin capsule, or it may be compressed into tablets. For oral therapeutic  
administration, the active ingredient may be incorporated with excipients and used in the  
30 form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups,  
wafers and the like.

[0098] Dosage regimens may be adjusted to provide the optimum desired response (e.g. a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or  
5 increased as indicated by exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage.

[0099] A physician or veterinarian having ordinary skill in the art can readily determine  
10 and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the antagonist, employed in the pharmaceutical composition, at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. In general, a suitable dose of a composition of the invention may be that amount  
15 of the compound which is the lowest dose effective to produce a therapeutic effect.

[0100] For therapeutic applications, the IL-11 muteins of the present invention or compositions containing those muteins are administered to a mammal, preferably a human, in a pharmaceutically acceptable dosage form such as those discussed above, including  
20 those that may be administered to a human intravenously as a bolus or by continuous infusion over a period of time.

[0101] In one aspect, the present invention contemplates a method for the treatment of a condition mediated by IL-11, the method comprising administering to said subject an  
25 effective amount of an IL-11 mutein of the present invention.

[0102] The IL-11 muteins of the present invention that are agonists, and compositions comprising such muteins, may be used in a method for the treatment of IL-11-mediated conditions where IL-11 exerts a positive effect.

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[0103] The IL-11 muteins of the present invention that are antagonists and compositions

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comprising such muteins may be used in a method for the treatment of IL-11-mediated conditions where IL-11 exerts a negative effect.

5 [0104] The IL-11 muteins of the present invention and compositions comprising such muteins may be used in a method of manufacture of a medicament for the treatment of IL-11-mediated conditions.

10 [0105] Particular IL-11 mutein antagonists of the present invention are IL-11 muteins having the amino acid sequences of SEQ ID NO:11, amino acids 10 to 178 of SEQ ID NO:11, amino acids 10 to 175 of SEQ ID NO:11, SEQ ID NO:12, amino acids 10 to 178 of SEQ ID NO:12 amino acids 10 to 175 of SEQ ID NO:12, SEQ ID NO:15, amino acids 10 to 178 of SEQ ID NO:15, amino acids 10 to 175 of SEQ ID NO:15, SEQ ID NO:19, amino acids 10 to 178 of SEQ ID NO:19, amino acids 10 to 175 of SEQ ID NO:19, SEQ ID NO:20, amino acids 10 to 178 of SEQ ID NO:20, amino acids 10 to 175 of SEQ ID NO:20, SEQ ID NO:21, amino acids 10 to 178 of SEQ ID NO:21 or amino acids 10 to 175 of SEQ ID NO:21 and which may be PEGylated. Particularly PEGylation is *via* attachment to the cysteine residue corresponding to position 147 of SEQ ID NO's 11, 12, 15, 19, 20 or 21.

20 [0106] The invention further contemplates the use of an IL-11 mutein in the manufacture of a medicament for the treatment of an IL-11-mediated condition.

[0107] The present invention is further described by the following non-limiting Examples.

- 30 -

**EXAMPLE 1*****IL-11 mutant proteins*****A. Recombinant production of soluble IL-11 mutant proteins**

5 [0108] The IL-11 mutants 1.21 (SEQ ID NO:10), 1B.382 (SEQ ID NO:17) and mIL-11-W147A were cloned into a modified version of the pET15b vector (Novagen Cat # 69661-3). The pET15b vector was modified by replacing the thrombin cleavage site and multiple cloning sites with *AscI* and *EcoRI* restriction sites, and by inserting an M13 origin of replication so the vector could be used as a phagemid.

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[0109] The corresponding N-terminal hexahistidine-tagged proteins were expressed in the *E.coli* strain BL21-CodonPlus [Registered trade mark] (DE3)-RIL *E.coli* (Stratagene cat # 230245). Typically, 400 mL shake-flask cultures in superbroth containing 2% v/v glucose and 100 µg/mL ampicillin were grown to an optical density (600 nm) of 0.5. Protein expression was then induced by the addition of isopropyl-β-D-thiogalactopyranoside to a final concentration of 200 µM, and the cultures were incubated with shaking at 37°C for a further 4 hours. The recombinant proteins were purified from the bacterial cells (lysed in 7 M guanidinium hydrochloride) using immobilized nickel ion affinity chromatography, and refolded by dialysis into PBS. The refolded samples were further dialyzed against 0.15% aqueous trifluoroacetic acid. In some cases samples were also purified by reverse phase HPLC using acetonitrile gradients in 0.15% v/v trifluoroacetic acid before lyophilization. Samples were reconstituted in a small volume of water prior to dilution with buffer.

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[0110] The affinity of the selected IL-11 mutants for IL-11Rα was determined in a competition ELISA experiment. 96 well plates coated with mIL-11Rα-Fc were incubated with a constant sub-saturating amount of the phage displayed IL-11 variants in the presence of different concentrations of soluble IL-11 proteins. After incubation for 2 hours at room temperature, the plates were washed and bound phage were then labeled with an anti-M13 polyclonal antibody conjugated to horseradish peroxidase. After the removal of excess antibody by washing with PBS containing 0.05% Tween 20, TMB substrate was added to each well and incubated for 10 minutes before the reaction was quenched by the

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addition of 2M phosphoric acid. Absorbance at 450 nm was then determined for each well by analysis on a microtitre plate reader.

[0111] The W147A mutation has no effect on IL-11R $\alpha$  binding but does prevent the recruitment of gp130 to the IL-11 receptor complex (Underhill-Day *et al*, 2003 *supra*) thereby preventing IL-11 signaling. W147A IL-11 is an antagonistic variant of IL-11.

[0112] Clear differences were observed between the affinities of the mIL-11-W147A and mutant proteins for binding to IL-11R $\alpha$ -Fc. Relative to W147A IL-11, clone 1.21 (SEQ ID NO:10) bound to IL-11R $\alpha$  with a 20-fold higher affinity, while clone 1B.382 (SEQ ID NO:17) also bound IL-11R $\alpha$  with a 20-fold higher affinity.

#### **B. *In vitro* activity of antagonist**

[0113] An IL-11 responsive Ba/F3 cell line was generated to test the ability of the mutant IL-11 proteins to block IL-11 bioactivity. Ba/F3 cells, a murine pro B-lymphocyte cell line which does not normally express IL-11R $\alpha$  or gp130 nor proliferate in response to IL-11 were stably transfected with constructs encoding wild-type murine IL-11R $\alpha$  and the co-receptor murine gp130 and selected by growth in media containing IL-11. Clonal cell lines were derived by limit dilution cloning. A number of stably transfected clones were analyzed for their dose-responsive proliferation (using a MTT assay) when cultured in the presence of IL-11 and one was selected for further work.

[0114] IL-11 responsive Ba/F3 cells stably transfected with murine IL-11R $\alpha$ /gp130 were seeded at  $3 \times 10^4$  cells/well in 50uL of Dulbecco's modified Eagle's medium containing 10% (v/v) fetal calf serum and increasing concentrations of mutant IL-11 proteins in the presence of a fixed, submaximal concentration of murine IL-11 (50 pM) in a total volume of 100 uL/well. After incubation for 48 hours, proliferation was measured colorimetrically at 570 nm using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma-Aldrich). Assays were always performed in duplicate and mean values for each assay point were then plotted.



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[0115] W147A IL-11 has been previously characterized as an antagonist of IL-11 bioactivity (Underhill-Day *et al*, 2003 *supra*). When IL-11R $\alpha$ /gp130 Ba/F3 cells were stimulated with a sub-maximal dose of IL-11, W147A IL-11 was able to inhibit cell proliferation in a dose-dependent manner. Several of the mutant IL-11 proteins were  
5 assayed for their ability to inhibit IL-11-induced proliferation and compared to W147A IL-11 (Table 4). The mutant IL-11 proteins were significantly more potent at blocking the proliferation induced IL-11 as measured in a standard MTT assay. Clones 1.21 (SEQ ID NO:10) and 1B.382 (SEQ ID NO:17) were both 20 to 30-fold more potent antagonists of IL-11 than W147A IL-11.

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**EXAMPLE 2*****PEGylated IL-11 muteins*****Production of PEGylated IL-11 muteins**

5 [0116] The mature protein sequence of murine IL-11 contains an amino acid sequence that can be cleaved by thrombin resulting in the removal of the first nine amino acids. A comparison of the IL-11 mutein 1.21 (SEQ ID NO:10) with and without the first nine amino acids showed identical activity and indicated that the first nine residues of murine IL-11 are not required for IL-11R $\alpha$  binding. The internal thrombin site was optimized by  
10 site directed mutagenesis to allow for efficient cleavage by mutation of residues 6 and 7 to Leu (L) and Val (V), respectively. For large scale production of the PEGylated IL-11 mutein of amino acids 10-178 of SEQ ID NO:12 the amino-terminal His-tag and the first nine residues of the modified mIL-11 sequence were removed by thrombin digestion.

15 [0117] For cleavage of the N-terminal hexahistidine tag, lyophilized samples of the relevant IL-11 mutein protein were re-suspended in thrombin cleavage buffer (150 mM NaCl, 2.5 mM CaCl<sub>2</sub>, 20 mM Tris.HCl pH 8.4) at a concentration of 0.5 mg/mL and treated with 5 units of thrombin/mg protein for 4 hours at room temperature. Under these conditions, thrombin efficiently cleaves murine derived IL-11 mutein at the optimized  
20 internal site between residues Arg<sup>9</sup>-Val<sup>10</sup> and the thrombin digested samples have an N-terminal sequence of Val<sup>10</sup>-Ser<sup>11</sup>-Ser<sup>12</sup>. Following treatment with thrombin the cleaved samples were purified by reverse phase HPLC as previously described.

**Site-specific PEGylation**

25 [0118] A limitation to the *in vivo* use of small proteins is their rapid clearance from circulation. One of the main routes of clearance is *via* filtration through the kidney, the efficiency of which is inversely proportional to the molecular weight. One strategy for reducing the *in vivo* clearance rate of small proteins is through chemical modification with polyethylene glycol (Tsutsumi *et al*, *Thjromb. Haemost.* 77.1:168-73, 1997), however, this  
30 can reduce or even eliminate the activity of a protein if attached at an inappropriate site.

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[0119] To improve the potential pharmacokinetic properties of a mutant IL-11 protein for *in vivo* use, a strategy was designed for site-specific modification of the mutant IL-11 proteins with a 40 kDa polyethylene glycol moiety. The absence of cysteine with the sequence of IL-11 was exploited to introduce a single unique Cys residue at position 147 by site directed mutagenesis. This provided a chemically reactive sidechain which could be site-specifically modified with a maleimide-derivatized PEG reagent. Moreover, the site of PEG attachment corresponds to site III on the surface of IL-11, and should not interfere with binding of the mutant IL-11 proteins to IL-11R $\alpha$ , or to the gp130 molecule which binds to the site II surface.

10

[0120] Modified forms of the mutant IL-11 proteins were generated containing a W147C mutation and the optimized internal thrombin site described above. The proteins were expressed in *E.coli* and purified and refolded as described in Example 1. The N-terminal His-tag and the first 9 N-terminal amino acids were then cleaved with thrombin as described above. The thrombin-treated samples were purified as described in Example 1 except that the samples was adjusted to pH 8.0 and reduced with 5 mM DTT prior to refolding in PBS containing 2mM EDTA and 2mM DTT.

15

[0121] Mutant IL-11 proteins containing an engineered Cys residue at the position corresponding to position 147 of SEQ ID NO:12 were then modified with 40 kDa maleimide-derivatized polyethylene glycol. Briefly, lyophilized thrombin-treated mutant IL-11 proteins were resuspended at a concentration of 5 mg/mL in 1 mM aqueous acetic acid containing 5 mM tris(2-carboxyethyl)phosphine, and mixed with 4 volumes of 12.5 mg/mL mPEG2-maleimide (Nektar Therapeutics cat #2D3YOTO1) in PBS. Reactions were incubated for 16 hours at room temperature and protein-PEG conjugates were then separated from unconjugated components by cation exchange chromatography on an SP Sepharose column, using a NaCl gradient in 20 mM sodium acetate, pH 5.5 buffer. Fractions containing the PEGylated products were pooled, dialyzed against 5 mM ammonium acetate buffer, pH 5.5, and then lyophilized.

20

25  
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[0122] A modified form of clone 1.21, containing a W147C mutation and an optimized internal thrombin site, was expressed in *E.coli*, and purified and refolded as described. The N-terminal His-tag and 9 amino acid residue fragment were cleaved with thrombin and then site-specifically PEGylated at Cys147 (where Cys147 is the position corresponding to position 147 of SEQ ID NO:12). Excess PEG reagent was removed by ion exchange chromatography. Analysis of the PEGylated and truncated modified form of clone 1.21 (referred to herein as  $\Delta$ 1.21) by SDS-PAGE showed a shift in apparent molecular weight consistent with attachment of a single 40 kDa PEG moiety.

10 [0123] The activity of  $\Delta$ 1.21 was tested in the IL-11R $\alpha$  binding ELISA and the Ba/F3 cell assay, and compared to the activity of non-PEGylated 1.21 (containing Ala at position 147) and with non-PEGylated W147A IL-11. In both assays, the activity of  $\Delta$ 1.21 was reduced relative to non-PEGylated 1.21. IL-11R $\alpha$  binding affinity was reduced approximately 5-fold, whilst the ability of  $\Delta$ 1.21 to antagonize IL-11-induced Ba/F3 cell proliferation was reduced approximately 10-fold. Moderate decreases in potency are commonly observed for PEGylated proteins and often result from a decrease in the rate of association between the protein and its target receptor. Despite the decrease in potency,  $\Delta$ 1.21 was nevertheless more potent than non-PEGylated W147A IL-11 in both assays.

20

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### EXAMPLE 3

#### *In vivo Half Life*

[0124] Female C57BL/6J mice (around 8 weeks old and 20 +/- 2g) were used to determine the *in vivo* half life of PEGylated mutein ( $\Delta$ 1.21) and non-PEGylated thrombin cleaved mutein 1.21 (i.e. amino acids 10-178 of SEQ ID NO:10). Each mouse received 1 IP injection. Mice were injected with the thrombin cleaved mutein 1.21 at a dose of 1mg/kg (20 ug per animal) or with an equivalent molar dose of  $\Delta$ 1.21 at a dose of 3.2 mg/kg (64 ug per animal). At an appropriate time following the IP injection, the mice were killed by CO<sub>2</sub> inhalation, followed by cervical dislocation, and blood collected by cardiac puncture. Sera was separated from the blood by incubation at 37°C for 1 hour and then overnight at 4°C before centrifugation to pellet the red blood cells. Blood was collected at 5 minute, 10 minutes, 30 minutes, 1 hour, 2 hours and 5 hours from mice injected with thrombin cleaved mutein 1.21 and at 10 minutes, 1 hour, 2 hours, 6 hours, 24 hours, 48 hours and 72 hours from mice injected with  $\Delta$ 1.21. A total of 4 mice were used for each time point.

[0125] The concentration of the thrombin cleaved mutein 1.21 was quantitated using a capture ELISA. Briefly, ELISA plates were coated with 2  $\mu$ g/ml mIL-11R $\alpha$ -Fc (50 $\mu$ l/well) [R&D systems] overnight at 4°C in PBS and the plates were then blocked with PBS containing 5% w/v skim milk (200 $\mu$ l/well) for 2 hours at room temperature. After washing the blocked plates with PBS containing 0.05% v/v Tween 20, serum samples were serially diluted in Tris buffered saline containing 1% w/v BSA and 0.05% v/v Tween 20 (TBS+BT) and added to the plates (100 $\mu$ l/well). The plates were incubated overnight at 4°C. Plates were washed with PBS containing 0.05% v/v Tween 20 after the overnight binding of the mutein in the serum to the mIL-11R $\alpha$ -Fc coated on the plate, and then incubated with polyclonal biotinylated anti-mIL-11 (50 $\mu$ l/well) [R&D systems, cat#BAF418] at 0.3 $\mu$ g/ml in TBS+BT for 2 hours at room temperature. After washing in PBS containing 0.05% v/v Tween 20 the plate was incubated with streptavidin-HRP (Sigma) [50 $\mu$ l/well] diluted 1000-fold in TBS+BT and incubated for 1 hour at room temperature. After washing in PBS containing 0.05% v/v Tween 20 TMB substrate was added to each well (100 $\mu$ l/well) and after 10 minutes incubation the reaction was stopped

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with 2M phosphoric acids (50 $\mu$ l/well) and the plate read at a wavelength of 450 nm using a microtitre plate reader. A standard curve was generated for both thrombin cleaved mutein 1.21 and  $\Delta$ 1.21 using known concentrations of proteins. These standard curves were then used to convert the ELISA data into nM values for thrombin cleaved mutein 1.21 and  
5  $\Delta$ 1.21 contained in the serum.

[0126] PEGylation of the mutein clearly improved the half-life. The highest concentration of unPEGylated thrombin cleaved mutein 1.21 in the serum was seen at the earliest time point of 5 minutes after administration and was in continual decline. Only minimal  
10 amounts of unPEGylated thrombin cleaved mutein 1.21 remained in the serum 5 hours after administration and was estimated to have a half-life of less than 1 hour. By comparison the highest concentration of  $\Delta$ 1.21 in the serum was seen at 6 hours post administration and was still present in measurable concentrations 72 hours post injection. The half-life of the  $\Delta$ 1.21 was estimated to be approximately 24 hours.

15

#### EXAMPLE 4

##### *PEGylated human IL-11 mutein*

[0127] A PEGylated human IL-11 mutein was prepared based on SEQ ID NO:11. This  
20 was expressed purified and refolded as described and then site-specifically PEGylated at Cys147 (numbering based on SEQ ID NO:11) using the general approach described above for the murine IL-11 mutein. Human IL-11 does not contain an internal thrombin site so the first 9 amino acids of the human IL-11 sequence as well as a tag sequence from the vector were retained to provide the mutein of SEQ ID NO:22.

25

[0128] The PEGylated human IL-11 mutein of SEQ ID NO:22 had equivalent activity to the pegylated mouse IL-11 mutein  $\Delta$ 1.21 described above in both ELISA and Ba/F3 assays.

30 [0129] Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to

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be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

5

**TABLE 3**  
*Cell assay data*

5

<b>Clone ID</b>	<b>Sequence</b>	<b>IC<sub>50</sub> (nM)</b>	<b>IC<sub>50</sub> (mutant)/ IC<sub>50</sub>(wild-type*)</b>
<b>wild-type*</b>		14	1.0
<b>1.21</b>	<sup>58</sup> P-A-I-D-Y <sup>62</sup>	0.54	26
<b>1B.382</b>	<sup>58</sup> F-M-Q-I-Q <sup>62</sup>	0.49	29



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**CLAIMS:**

1. An IL-11 mutein wherein the amino acid sequence AMSAG at position 58 to 62 of wild-type mammalian IL-11 is replaced with the amino acid sequence PAIDY or FMQIQ.
2. The IL-11 mutein of Claim 1 wherein the amino acid sequence AMSAG is replaced by PAIDY.
3. The IL-11 mutein of Claim 1 wherein the amino acid sequence AMSAG is replaced by FMQIQ.
4. The IL-11 mutein of Claim 1 or 2 or 3 comprising a further mutation which inhibits or reduces its binding to gp130.
5. The IL-11 mutein of Claim 4 wherein the further mutation is an amino acid substitution of the tryptophan (W) at amino acid position 147 of the wild-type IL-11.
6. The IL-11 mutein of Claim 5 wherein the tryptophan (W) is substituted by an alanine (A) or cysteine (C).
7. The IL-11 mutein of Claim 1 comprising an amino acid sequence selected from SEQ ID NO:4, amino acids 10 to 178 of SEQ ID NO:4, amino acids 10 to 175 of SEQ ID NO:4, SEQ ID NO:5, amino acids 10 to 178 of SEQ ID NO:5, amino acids 10 to 175 of SEQ ID NO:5, SEQ ID NO:6, amino acids 10 to 178 of SEQ ID NO:6, amino acids 10 to 175 of SEQ ID NO:6, SEQ ID NO:7, amino acids 10 to 178 of SEQ ID NO:7, amino acids 10 to 175 of SEQ ID NO:7, SEQ ID NO:8, amino acids 10 to 178 of SEQ ID NO:8, amino acids 10 to 175 of SEQ ID NO:8, SEQ ID NO:13, amino acids 10 to 178 of SEQ ID NO:13 and amino acids 10 to 175 of SEQ ID NO:13.
8. The IL-11 mutein of Claim 1 comprising an amino acid sequence selected from SEQ ID NO:9, amino acids 10 to 178 of SEQ ID NO:9, amino acids 10 to 175 of SEQ ID

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NO:9, SEQ ID NO:10, amino acids 10 to 178 of SEQ ID NO:10, amino acids 10 to 175 of SEQ ID NO:10, SEQ ID NO:11, amino acids 10 to 178 of SEQ ID NO:11, amino acids 10 to 175 of SEQ ID NO:11, SEQ ID NO:12, amino acids 10 to 178 of SEQ ID NO:12, amino acids 10 to 175 of SEQ ID NO:12, SEQ ID NO:14, amino acids 10 to 178 of SEQ ID NO:14, amino acids 10 to 175 of SEQ ID NO:14, SEQ ID NO:15, amino acids 10 to 178 of SEQ ID NO:15, amino acids 10 to 175 of SEQ ID NO:15, SEQ ID NO:16, amino acids 10 to 178 of SEQ ID NO:16, amino acids 10 to 175 of SEQ ID NO:16, SEQ ID NO:17, amino acids 10 to 178 of SEQ ID NO:17, amino acids 10 to 175 of SEQ ID NO:17, SEQ ID NO:18, amino acids 10 to 178 of SEQ ID NO:18, amino acids 10 to 175 of SEQ ID NO:18, SEQ ID NO:19, amino acids 10 to 178 of SEQ ID NO:19, amino acids 10 to 175 of SEQ ID NO:19, SEQ ID NO:20, amino acids 10 to 178 of SEQ ID NO:20, amino acids 10 to 175 of SEQ ID NO:20, SEQ ID NO:21, amino acids 10 to 178 of SEQ ID NO:21 and amino acids 10 to 175 of SEQ ID NO:21.

9. The IL-11 mutein of Claim 1 or 7 or 8 wherein said IL-11 mutein is PEGylated.
10. A pharmaceutical composition comprising the IL-11 mutein of any one of Claims 1 to 9 and one or more pharmaceutical acceptable carriers, diluents and/or excipients.
11. A method for the treatment of an IL-11-mediated condition, said method comprising administering to said subject an effective amount of an IL-11 mutein as claimed in any one of Claims 1 to 9.
12. Use of an IL-11 mutein of any one of Claims 1 to 9 in the manufacture of a medicament in the treatment of an IL-11-mediated condition.

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**Human: Mature IL-11 Sequence**

(SEQ ID NO:1)

PGPPP GPPRV .SPDPRAELDS .TVLLTRSLLA .DTRQLAAQLR .DKFPADGDHN .LDSLPTLAMS  
AGALGALQLP GVLTRLRADL LSYLRHVQWL RRAGGSSLKT LEPELGTLQA RLDRLLRRLQ  
LLMSRLALPQ PPPDPPAPPL APPSSAWGGI RAAHAILGGL HLTLDWAVRG LLLLKTRL

**Murine: Mature IL-11 Sequence**

(SEQ ID NO:2)

PGPPAGSPRV SSDPRADLDS AVLLTRSLLA DTRQLAAQMR DKFPADGDHS LDSLPTLAMS  
AGTLGSLQLP GVLTRLRVDL MSYLRHVQWL RRAGGSSLKT LEPELGTLQA RLERLLRRLQ  
LLMSRLALPQ AAPDQPIPL GPPASAWGSI RAAHAILGGL HLTLDWAVRG LLLLKTRL

**Macaque: Mature IL-11 Sequence**

(SEQ ID NO:3)

PGPPPGSPRA SPDPRAELDS TVLLTRSLLE DTRQLTIQLK DKFPADGDHN LDSLPTLAMS  
AGALGALQLP SVLTRLRADL LSYLRHVQWL RRAMGSSLKT LEPELGTLQT RLDRLLRRLQ  
LLMSRLALPQ LPPDPPAPPL APPSSTWGGI RAAHAILGGL HLTLDWAVRG LLLLKTRL

**Figure 1a**

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**Macaque: PAIDY IL-11 Sequence**

(SEQ ID NO:4)

PGPPPGSPRA SPDPRAELDS TVLLTRSLLE DTRQLTIQLK DKFPADGDHN LDSLPTLP  
DYALGALQLP SVLTRLRADL LSYLRHVQWL RRAMGSSLKT LEPELGTLQT RLDRLLRRLQ  
LLMSRLALPQ LPPDPPAPPL APPSSTWGGI RAAHAILGGL HLTLDWAVRG LLLLKTRL

**Human: PAIDY IL-11 Mutein**

(SEQ ID NO:5)

PGPPPGPPRV .SPDPRAELDS .TVLLTRSLLA .DTRQLAAQLR .DKFPADGDHN .LDSLPTLP  
DYALGALQLP GVLTRLRADL LSYLRHVQWL RRAGGSSLKT LEPELGTLQA RLDRLLRRLQ  
LLMSRLALPQ PPPDPPAPPL APPSSAWGGI RAAHAILGGL HLTLDWAVRG LLLLKTRL

**Murine: PAIDY IL-11 Mutein**

(SEQ ID NO:6)

PGPPAGSPRV SSDPRADLDS AVLLTRSLLA DTRQLAAQMR DKFPADGDHS LDSLPTLP  
DYTLGSLQLP GVLTRLRVDL MSYLRHVQWL RRAGGPSLKT LEPELGALQA RLRLLRRLQ  
LLMSRLALPQ AAPDQPVIPL GPPASAWGSI RAAHAILGGL HLTLDWAVRG LLLLKTRL

**Figure 1b**



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**Human: FMQIQ IL-11 Mutein**

(SEQ ID NO:7)

PGPPP GPPRV .SPDPRAELDS .TVLLTRSLLA .DTRQLAAQLR .DKFPADGDHN .LDSLPTLFMQ  
IQALGALQLP GVLTRLRADL LSYLRHVQWL RRAGGSSLKT LEPELGTLQA RLDRLLRRLQ  
LLMSRLALPQ PPPDPPAPPL APPSSAWGGI RAAHAILGGL HLTLDWAVRG LLLLKTRL

**Murine: FMQIQ IL-11 Mutein**

(SEQ ID NO:8)

PGPPAGSPRV SSDPRADLDS AVLLTRSLLA DTRQLAAQMR DKFPADGDHS LDSLPTLFMQ  
IQTLGSLQLP GVLTRLRVDL MSYLRHVQWL RRAGGSSLKT LEPELGALQA RLERLLRRLQ  
LLMSRLALPQ AAPDQPIPL GPPASAWGSI RAAHAILGGL HLTLDWAVRG LLLLKTRL

**Macaque: FMQIQ IL-11 Sequence**

(SEQ ID NO:13)

PGPPPGSPRA SPDPRAELDS TVLLTRSLLE DTRQLTIQLK DKFPADGDHN LDSLPTLFMQ  
IQALGALQLP SVLTRLRADL LSYLRHVQWL RRAMGSSLKT LEPELGTLQT RLDRLLRRLQ  
LLMSRLALPQ LPPDPPAPPL APPSSTWGGI RAAHAILGGL HLTLDWAVRG LLLLKTRL

**Figure 1c**

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**Human: PAIDY & W147A IL-11 Mutein**

(SEQ ID NO:9)

PGPPPGPPRV .SPDPRAELDS .TVLLTRSLLA .DTRQLAAQLR .DKFPADGDHN .LDSLPTLP  
DIALGALQLP GVLTRLRADL LSYLRHVQWL RRAGGSSLKT LEPGLGTLQA RLDRLRRRLQ  
LLMSRLALPQ PPPDPPAPPL APPSSAAGGI RAAHAILGGL HLTLDWAVRG LLLLKTRL

**Murine: PAIDY & W147A IL-11 Mutein**

(SEQ ID NO:10)

PGPPAGSPRV SSDPRADLDS AVLLTRSLLA DTRQLAAQMR DKFPADGDHS LDSLPTLP  
DYTLGSLQLP GVLTRLRVLD MSYLRHVQWL RRAGGSSLKT LEPGLGALQA RLRLRRRLQ  
LLMSRLALPQ AAPDQPIPL GPPASAAGSI RAAHAILGGL HLTLDWAVRG LLLLKTRL

**Macaque: PAIDY & W147A IL-11 Sequence**

(SEQ ID NO:14)

PGPPPGSPRA SPDPRAELDS TVLLTRSLLE DTRQLTIQLK DKFPADGDHN LDSLPTLP  
DIALGALQLP SVLTRLRADL LSYLRHVQWL RRAMGSSLKT LEPGLGTLQT RLDRLRRRLQ  
LLMSRLALPQ LPPDPPAPPL APPSSTAGGI RAAHAILGGL HLTLDWAVRG LLLLKTRL

**Figure 1d**

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**Human: PAIDY & W147C IL-11 Mutein**

(SEQ ID NO:11)

PGPPPGPRV .SPDPRAELDS .TVLLTRSLLA .DTRQLAAQLR .DKFPADGDHN .LDSLPTLP  
DIALGALQLP GVLTRLRADL LSYLRHVQWL RRAGGSSLKT LEPELGTLQA RLDRLRRRLQ  
LLMSRLALPQ PPPDPPAPPL APPSSACGGI RAAHAILGGL HLTLDWAVRG LLLLKTRL

**Murine: PAIDY & W147C IL-11 Mutein**

(SEQ ID NO:12)

PGPPAGSPRV SSDPRADLDS AVLLTRSLLA DTRQLAAQMR DKFPADGDHS LDSLPTLP  
DYTLGSLQLP GVLTRLRVDL MSYLRHVQWL RRAGGSSLKT LEPELGTALQA RLERLLRRRLQ  
LLMSRLALPQ AAPDQVPIPL GPPASACGSI RAAHAILGGL HLTLDWAVRG LLLLKTRL

**Macaque: PAIDY & W147C IL-11 Sequence**

(SEQ ID NO:15)

PGPPPGSPRA SPDPRAELDS TVLLTRSLLE DTRQLTIQLK DKFPADGDHN LDSLPTLP  
DIALGALQLP SVLTRLRADL LSYLRHVQWL RRAMGSSLKT LEPELGTLQT RLDRLRRRLQ  
LLMSRLALPQ LPPDPPAPPL APPSSTCGGI RAAHAILGGL HLTLDWAVRG LLLLKTRL

**Figure 1e**

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**Human: FMQIQ & W147A IL-11 Mutein**

(SEQ ID NO:16)

PGPPPGPPRV .SPDPRAELDS .TVLLTRSLLA .DTRQLAAQLR .DKFPADGDHN .LDSLPTLFMQ  
IQALGALQLP GVLTRLRADL LSYLRHVQWL RRAGGSSLKT LEPELGTLQA RLDRLLRRLQ  
LLMSRLALPQ PPPDPPAPPL APPSSAAGGI RAAHAILGGL HLTLDWAVRG LLLLKTRL

**Murine: FMQIQ & W147A IL-11 Mutein**

(SEQ ID NO:17)

PGPPAGSPRV SSDPRADLDS AVLLTRSLLA DTRQLAAQMR DKFPADGDHS LDSLPTLFMQ  
IQTIGSLQLP GVLTRLRVDL MSYLRHVQWL RRAGGSSLKT LEPELGALQA RLRLLRRLQ  
LLMSRLALPQ AAPDQPIPL GPPASAAGSI RAAHAILGGL HLTLDWAVRG LLLLKTRL

**Macaque: FMQIQ & W147A IL-11 Sequence**

(SEQ ID NO:18)

PGPPPGSPRA SPDPRAELDS TVLLTRSLLE DTRQLTIQLK DKFPADGDHN LDSLPTLFMQ  
IQALGALQLP SVLTRLRADL LSYLRHVQWL RRAMGSSLKT LEPELGTLQT RLDRLLRRLQ  
LLMSRLALPQ LPPDPPAPPL APPSSTAGGI RAAHAILGGL HLTLDWAVRG LLLLKTRL

**Figure 1f**

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**Human: FMQIQ & W147C IL-11 Mutein**

(SEQ ID NO:19)

PGPPPGPPRV .SPDPRAELDS .TVLLTRSLLA .DTRQLAAQLR .DKFPADGDHN .LDSLPTLFMQ  
 IQALGALQLP GVLTRLRADL LSYLRHVQWL RRAGGSSLKT LEPELGTLOA RLDRLLRRLQ  
 LLMSRLALPQ PPPDPPAPPL APPSSACGGI RAAHAILGGL HLTLDWAVRG LLLLKTRL

**Murine: FMQIQ & W147C IL-11 Mutein**

(SEQ ID NO:20)

PGPPAGSPRV SSDPRADLDS AVLLTRSLLA DTRQLAAQMR DKFPADGDHS LDSLPTLFMQ  
 IQTLGSLQLP GVLTRLRVDL MSYLRHVQWL RRAGGSSLKT LEPELGTLOA RLDRLLRRLQ  
 LLMSRLALPQ AAPDQPIPL GPPASACGSI RAAHAILGGL HLTLDWAVRG LLLLKTRL

**Macaque: FMQIQ & W147C IL-11 Sequence**

(SEQ ID NO:21)

PGPPPGSPRA SPDPRAELDS TVLLTRSLLE DTRQLTIQLK DKFPADGDHN LDSLPTLFMQ  
 IQALGALQLP SVLTRLRADL LSYLRHVQWL RRAMGSSLKT LEPELGTLOT RLDRLLRRLQ  
 LLMSRLALPQ LPPDPPAPPL APPSSTCGGI RAAHAILGGL HLTLDWAVRG LLLLKTRL

**Human: N-terminally tagged PAIDY & W147C IL-11 Sequence**

(SEQ ID NO:22)

MGSHHHHHHG ARQPGPPGP PRVSPDPRAE LDSTVLLTRS LLADTRQLAA QLRDKFPADG  
 DHNLDSLPTL PAIDYALGAL QLPGLVTRLR ADLLSYLRHV QWLRRAGGSS LKTLEPELGT  
 LQARLDRLLR RLQLLMSRLA LPQPPDPPA PPLAPPSSAC GGIRAAHAIL GGLHLTLDWA  
 VRGLLLKTR L

**Figure 1g**

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2008/001587

A. CLASSIFICATION OF SUBJECT MATTER		
Int. Cl.		
<i>C07K 14/54</i> (2006.01)	<i>A61P 15/16</i> (2006.01)	<i>A61P 19/08</i> (2006.01)
<i>A61K 38/20</i> (2006.01)	<i>A61P 15/18</i> (2006.01)	<i>A61P 19/10</i> (2006.01)
ACTION DATE: 26 NOVEMBER 2008		
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)		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
GenomeQuest: Sequence search based on SEQ ID NO: 4-8 and 13		
CA, Medline & WPIDS & keywords: Interleukin-11, antagonist, binding and like terms		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CZUPRYN, M.J., et al. Structure-Function Relationships in Human Interleukin-11. Journal of Biological Chemistry. 1995. vol 270, pages 978-985. See whole document, especially page 979- page 980 section 'Modification of Methionine Residues'.	
A	UNDERHILL-DAY, N., et al. Functional Characterisation of W147A: A High-Affinity Interleukin-11 Antagonist. Endocrinology. 2003. vol. 144, pages 3406-3414. See whole document especially figure 5, page 3413, column 1, lines 27-34.	
<input type="checkbox"/> Further documents are listed in the continuation of Box C <input type="checkbox"/> See patent family annex		
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"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		
Date of the actual completion of the international search 27 November 2008	Date of mailing of the international search report 7 - JAN 2009	
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