effector function of an Fc region containing protein are known in the art and/or described herein.

In one example, the Fc region is an IgG4 Fc region (i.e., from an IgG4 constant region), e.g., a human IgG4 Fc region. Sequences of suitable IgG4 Fc regions will be apparent to the skilled person and/or available in publically available databases (e.g., available from National Center for Biotechnology Information).

In one example, the constant region is a stabilized IgG4 constant region. The term "stabilized IgG4 constant region" will be understood to mean an IgG4 constant region that has been modified to reduce Fab arm exchange or the propensity to undergo Fab arm exchange or formation of a half-antibody or a propensity to form a half antibody. "Fab arm exchange" refers to a type of protein modification for human IgG4, in which an IgG4 heavy chain and attached light chain (half-molecule) is swapped for a heavy-light chain pair from another IgG4 molecule. Thus, IgG4 molecules may acquire two distinct Fab arms recognizing two distinct antigens (resulting in bispecific molecules). Fab arm exchange occurs naturally *in vivo* and can be induced *in vitro* by purified blood cells or reducing agents such as reduced glutathione. A "half antibody" forms when an IgG4 antibody dissociates to form two molecules each containing a single heavy chain and a single light chain.

In one example, a stabilized IgG4 constant region comprises a proline at position 241 of the hinge region according to the system of Kabat (Kabat *et al.*, Sequences of Proteins of Immunological Interest Washington DC United States Department of Health and Human Services, 1987 and/or 1991). This position corresponds to position 228 of the hinge region according to the EU numbering system (Kabat *et al.*, Sequences of Proteins of Immunological Interest Washington DC United States Department of Health and Human Services, 2001 and Edelman *et al., Proc. Natl. Acad. USA, 63*, 78-85, 1969). In human IgG4, this residue is generally a serine. Following substitution of the serine for proline, the IgG4 hinge region comprises a sequence CPPC. In this regard, the skilled person will be aware that the "hinge region" is a proline-rich portion of an antibody heavy chain constant region that links the Fc and Fab regions that confers mobility on the two Fab arms of an antibody. The hinge region includes cysteine residues which are involved in inter-heavy chain disulfide bonds. It is generally defined as stretching from Glu226 to Pro243 of human IgG1 according to the numbering system of Kabat. Hinge regions of other IgG isotypes may be aligned with the IgG1 sequence by placing the first and last cysteine residues forming inter-heavy chain disulphide (S-S) bonds in the same positions (see for example WO2010/080538).

Additional examples of stabilized IgG4 antibodies are antibodies in which arginine at position 409 in a heavy chain constant region of human IgG4 (according to the EU numbering system) is substituted with lysine, threonine, methionine, or leucine (e.g., as described in WO2006/033386). The Fc region of the constant region may additionally or alternatively comprise a residue selected from the group consisting of: alanine, valine, glycine, isoleucine and leucine at the position corresponding to 405 (according to the EU numbering system). Optionally, the hinge region comprises a proline at position 241 (i.e., a CPPC sequence) (as described above).

In another example, the Fc region is a region modified to have reduced effector function, i.e., a "non-immunostimulatory Fc region". For example, the Fc region is an IgG1 Fc region comprising a substitution at one or more positions selected from the group consisting of 268, 309, 330 and 331. In another example, the Fc region is an IgG1 Fc region comprising one or more of the following changes E233P, L234V, L235A and deletion of G236 and/or one or more of the following changes A327G, A330S and P331S (Armour *et al., Eur J Immunol. 29*:2613-2624, 1999; Shields *et al., J Biol Chem. 276(9)*:6591-604, 2001). Additional examples of non-immunostimulatory Fc regions are described, for example, in Dall'Acqua *et al., J Immunol. 177* : 1129-1138 2006; and/or Hezareh *J Virol ;75*: 12161-12168, 2001).

In another example, the Fc region is a chimeric Fc region, e.g., comprising at least one C_H2 domain from an IgG4 antibody and at least one C_H3 domain from an IgG1 antibody, wherein the Fc region comprises a substitution at one or more amino acid positions selected from the group consisting of 240, 262, 264, 266, 297, 299, 307, 309, 323, 399, 409 and 427 (EU numbering) (e.g., as described in WO2010/085682). Exemplary substitutions include 240F, 262L, 264T, 266F, 297Q, 299A, 299K, 307P, 309K, 309M, 309P, 323F, 399S, and 427F.

Enhancing Effector Function

In one example, an IL-11Rα-binding protein of the present disclosure may induce effector function or enhanced effector function.

In the context of the present disclosure, "effector functions" refer to those biological activities mediated by cells or proteins that bind to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody that result in killing of a cell. Examples of effector functions induced by antibodies include: complement dependent cytotoxicity; antibody-dependent-cell-mediated cytotoxicity (ADCC); antibody-dependent-cell-phagocytosis (ADCP); and B-cell activation.

In one example, an IL-11Rα-binding protein of the present disclosure binds to IL-11Rα on the surface of a cell in such a manner that it is capable of inducing an effector function, such as, ADCC or CDC.

For example, the IL-11Rα-binding protein remains bound to the IL-11Rα on the surface of the cell for a time sufficient to induce an effector function, such as ADCC and/or CDC.

In one example, an IL-11Rα-binding protein of the present disclosure is capable of inducing enhanced effector function, e.g., by virtue of a modified Fc region or by virtue of comprising a region capable of binding to an immune effector cell. For example, the level of effector function is increased compared to the level induced by a human IgG1 or IgG3 Fc region. Enhancing effector function induced by a IL-11Rα-binding protein of the disclosure may result in enhanced therapeutic or prophylactic effects, e.g., by not only blocking the action of IL-11Rα but also by killing or depleting cells causing a condition, e.g., by killing auto-reactive T cells.

In one example, the Fc region of an IL-11Rα-binding protein of the disclosure is modified to increase the level of effector function it is capable of inducing compared to the Fc region without the modification. Such modifications can be at the amino acid level and/or the secondary structural level and/or the tertiary structural level and/or to the glycosylation of the Fc region.

The skilled addressee will appreciate that greater effector function may be manifested in any of a number of ways, for example as a greater level of effect, a more sustained effect or a faster rate of effect.

In one example, the Fc region comprises one or more amino acid modifications that increase its ability to induce enhanced effector function. In one example, the Fc region binds with greater affinity to one or more FcγRs, such as FcγRIII. In one example, the Fc region comprise at least one amino acid substitution at a position selected from the group consisting of: 230, 233, 234, 235, 239, 240, 243, 264, 266, 272, 274, 275, 276, 278, 302, 318, 324, 325, 326, 328, 330, 332, and 335, numbered according to the EU index of Kabat. In one example, the Fc region comprises the following amino acid substitutions S239D/I332E, numbered according to the EU index of Kabat. This Fc region has about 14 fold increase in affinity for FcγRIIIa compared to a wild-type Fc region and about 3.3 increased ability to induce ADCC compared to a wild-type Fc region. In one example, the Fc region comprises the following amino acid substitutions S239D/A330L/I332E, numbered according to the EU index of Kabat. This Fc region has about 138 fold increase in affinity for FcγRIIIa compared to a wild-type Fc region and about 323 fold increased ability to induce ADCC compared to a wild-type Fc region.

Additional amino acid substitutions that increase ability of a Fc region to induce effector function are known in the art and/or described, for example, in US6737056 or US7317091.

In one example, the glycosylation of the Fc region is altered to increase its ability to induce enhanced effector function. In this regard, native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the C_H2 domain of the Fc region. The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some examples, Fc regions according to the present disclosure comprise a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region, i.e., the Fc region is "afucosylated". Such variants may have an improved ability to induce ADCC. Methods for producing afucosylated antibodies include, expressing the antibody or antigen binding fragment thereof in a cell line incapable of expressing α-1,6-fucosyltransferase (FUT8) (e.g., as described in Yumane-Ohnuki *et al., Biotechnol. Bioengineer. 87*: 614-622, 2004), expressing the antibody or antigen binding fragment thereof in cells expressing a small interfering RNA against FUT8 (e.g., as described in Mori *et al., Biotechnol. Bioengineer., 88*: 901-908, 2004), expressing the antibody or antigen binding fragment thereof in cells incapable of expressing guanosine diphosphate (GDP)-mannose 4,6-dehydratase (GMD) (e.g., as described in Kanda *et al., J. Biotechnol., 130*: 300-310, 2007). The present disclosure also contemplates the use of proteins having a reduced level of fucosylation, e.g., produced using a cell line modified to express β—(1,4)-*N*-acetylglucosaminyltransferase III (GnT-III) (e.g., as described in Umāna *et al., Nat. Biotechnol. 17*: 176-180, 1999).

Other methods include the use of cell lines which inherently produce antibodies capable of inducing enhanced Fc-mediated effector function (e.g. duck embryonic derived stem cells for the production of viral vaccines, WO2008/129058; Recombinant protein production in avian EBX® cells, WO 2008/142124).

IL-11Rα-binding proteins of the present disclosure also include those with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region is bisected by GlcNAc. Such proteins may have reduced fucosylation and/or improved ADCC function. Examples of such proteins are described, e.g., in US6602684 and US20050123546.

IL-11Rα-binding proteins with at least one galactose residue in the oligosaccharide attached to the Fc region are also contemplated. Such proteins may have improved CDC function. Such proteins are described, e.g., in WO1997/30087 and WO1999/22764.

IL-11Rα-binding proteins can also comprise a Fc region capable of inducing enhanced levels of CDC. For example, hybrids of IgG1 and IgG3 produce antibodies having enhanced CDC activity (Natsume *et al., Cancer Res. 68*: 3863-3872, 2008).

IL-11Rα-binding proteins can also or alternatively be fused to or conjugated to proteins (e.g., antibody variable regions) that bind to immune effector cells, e.g., by virtue of binding to CD3 or CD16.

Methods for determining effector function are known in the art. In one example, the level of ADCC activity is assessed using a ^{51}Cr release assay, an europium release assay or a ^{35}S release assay. In each of these assays, cells expressing IL-11Rα are cultured with one or more of the recited compounds for a time and under conditions sufficient for the compound to be taken up by the cell. In the case of a ^{35}S release assay, the cells can be cultured with ^{35}S-labeled methionine and/or cysteine for a time sufficient for the labeled amino acids to be incorporated into newly synthesized proteins. Cells are then cultured in the presence or absence of the IL-11Rα-binding protein and in the presence of immune effector cells, e.g., PBMCs and/or NK cells. The amount of ^{51}Cr, europium and/or ^{35}S in cell culture medium is then detected, and an increase in the presence of the protein compared to in the absence of protein indicates that the binding molecule/agent has effector function. Exemplary publications disclosing assays for assessing the level of ADCC induced by a protein include Hellstrom *et al. Proc. Natl Acad. Sci. USA 83*: 7059-7063, 1986 and Bruggemann *et al., J. Exp. Med. 166*: 1351-1361, 1987.

Other assays for assessing the level of ADCC induced by a protein include ACTI™ nonradioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. CA, USA) or CytoTox 96® non-radioactive cytotoxicity assay (Promega, WI, USA).

Alternatively, or additionally, effector function of an IL-11Rα-binding protein is assessed by determining its affinity for one or more FcγRs, e.g., as described in US7317091.

C1q binding assays may also be carried out to confirm that the IL-11Rα-binding protein is able to bind C1q and may induce CDC. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro *et al., J. Immunol. Methods 202*: 163, 1996).

Additional Modifications

The present disclosure also contemplates additional modifications to an antibody or IL-11Rα-binding protein comprising an Fc region or constant region.

For example, the antibody comprises one or more amino acid substitutions that increase the half-life of the protein. For example, the antibody comprises a Fc region comprising one or more amino acid substitutions that increase the affinity of the Fc

region for the neonatal Fc region (FcRn). For example, the Fc region has increased affinity for FcRn at lower pH, e.g., about pH 6.0, to facilitate Fc/FcRn binding in an endosome. In one example, the Fc region has increased affinity for FcRn at about pH 6 compared to its affinity at about pH 7.4, which facilitates the re-release of Fc into blood following cellular recycling. These amino acid substitutions are useful for extending the half life of a protein, by reducing clearance from the blood.

Exemplary amino acid substitutions include T250Q and/or M428L or T252A, T254S and T266F or M252Y, S254T and T256E or H433K and N434F according to the EU numbering system. Additional or alternative amino acid substitutions are described, for example, in US20070135620 or US7083784.

Exemplary IL-11Rα-Binding Proteins

Exemplary variable region containing IL-11Rα-binding proteins produced by the inventors are described in Table 1.

Table 1: Sequences of exemplary IL-11Rα-binding proteins

	Antibody Name	**V_L amino acid SEQ ID NO**	**V_H amino acid SEQ ID NO**
1	8E2	5	37
2	TS-303	6	37
3	TS-305	7	37
4	TS-306	8	37
5	TS-307	9	37
6	TS-310	10	37
7	TS-311	11	37
8	TS-312	12	37
9	TS-313	13	37
10	TS-322	14	37
11	TS-2	15	37
12	TS-4	16	37
13	TS-6	17	37
14	TS-7	18	37
15	TS-9	19	37
16	TS-13	20	37
17	TS-14	21	37
18	TS-17	22	37

19	TS-20	23	37
20	TS-21	24	37
21	TS-22	25	37
22	TS-29	26	37
23	TS-32	27	37
24	TS-49	28	37
25	TS-51	29	37
26	TS-55	30	37
27	TS-57	31	37
28	TS-58	32	37
29	TS-63	33	37
30	TS-64	34	37
31	TS-66	5	38
32	TS-69	5	39
33	TS-71	5	40
34	TS-76	5	41
35	TS-79	5	42
36	TS-82	5	43
37	TS-88	5	44
38	TS-89	5	45
39	TS-91	5	46
40	TS-92	5	47
41	TS-97	5	48
42	TS-101	5	49
43	TS-103	5	50
44	TS-104	5	51
45	TS-107	5	52
46	TS-108	5	53
47	TS-115	5	54
48	TS-129	5	55
49	TS-133	5	56
50	TS-134	5	57
51	TS-135	5	58
52	TS-136	5	59
53	TS-140	5	60
54	TS-143	5	61
55	TS-151	5	62

56	TS-156	5	63
57	TS-213	5	64
58	TS-214	5	65
59	TS-215	5	66
60	TS-218	5	67
61	TS-221	5	68
62	TS-222	5	69
63	TS-224	5	70
64	8E4	73	74
65	8D10	75	76

Protein Production

In one example, an IL-11Rα-binding protein described herein according to any example is produced by culturing a hybridoma under conditions sufficient to produce the protein, e.g., as described herein and/or as is known in the art.

Recombinant Expression

In another example, an IL-11Rα-binding protein described herein according to any example is recombinant.

In the case of a recombinant protein, nucleic acid encoding same can be cloned into expression constructs or vectors, which are then transfected into host cells, such as *E. coli* cells, yeast cells, insect cells, or mammalian cells, such as simian COS cells, Chinese Hamster Ovary (CHO) cells, human embryonic kidney (HEK) cells, or myeloma cells that do not otherwise produce the protein. Exemplary cells used for expressing a protein are CHO cells, myeloma cells or HEK cells. Molecular cloning techniques to achieve these ends are known in the art and described, for example in Ausubel *et al.,* (editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present) or Sambrook *et al.,* Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press (1989). A wide variety of cloning and *in vitro* amplification methods are suitable for the construction of recombinant nucleic acids. Methods of producing recombinant antibodies are also known in the art, see, e.g., US4816567 or US5530101.

Following isolation, the nucleic acid is inserted operably linked to a promoter in an expression construct or expression vector for further cloning (amplification of the DNA) or for expression in a cell-free system or in cells.

As used herein, the term "promoter" is to be taken in its broadest context and includes the transcriptional regulatory sequences of a genomic gene, including the TATA box or initiator element, which is required for accurate transcription initiation, with or without additional regulatory elements (e.g., upstream activating sequences, transcription factor binding sites, enhancers and silencers) that alter expression of a nucleic acid, e.g., in response to a developmental and/or external stimulus, or in a tissue specific manner. In the present context, the term "promoter" is also used to describe a recombinant, synthetic or fusion nucleic acid, or derivative which confers, activates or enhances the expression of a nucleic acid to which it is operably linked. Exemplary promoters can contain additional copies of one or more specific regulatory elements to further enhance expression and/or alter the spatial expression and/or temporal expression of said nucleic acid.

As used herein, the term "operably linked to" means positioning a promoter relative to a nucleic acid such that expression of the nucleic acid is controlled by the promoter.

Many vectors for expression in cells are available. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, a sequence encoding a protein (e.g., derived from the information provided herein), an enhancer element, a promoter, and a transcription termination sequence. The skilled artisan will be aware of suitable sequences for expression of a protein. Exemplary signal sequences include prokaryotic secretion signals (e.g., pelB, alkaline phosphatase, penicillinase, Ipp, or heat-stable enterotoxin II), yeast secretion signals (e.g., invertase leader, α factor leader, or acid phosphatase leader) or mammalian secretion signals (e.g., herpes simplex gD signal).

Exemplary promoters active in mammalian cells include cytomegalovirus immediate early promoter (CMV-IE), human elongation factor 1-α promoter (EF1), small nuclear RNA promoters (U1a and U1b), α-myosin heavy chain promoter, Simian virus 40 promoter (SV40), Rous sarcoma virus promoter (RSV), Adenovirus major late promoter, β-actin promoter; hybrid regulatory element comprising a CMV enhancer/ β-actin promoter or an immunoglobulin promoter or active fragment thereof. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture; baby hamster kidney cells (BHK, ATCC CCL 10); or Chinese hamster ovary cells (CHO).

Typical promoters suitable for expression in yeast cells such as for example a yeast cell selected from the group comprising *Pichia pastoris, Saccharomyces*

cerevisiae and *S. pombe*, include, but are not limited to, the *ADH1* promoter, the *GAL1* promoter, the *GAL4* promoter, the *CUP1* promoter, the *PHO5* promoter, the *nmt* promoter, the *RPR1* promoter, or the *TEF1* promoter.

Means for introducing the isolated nucleic acid or expression construct comprising same into a cell for expression are known to those skilled in the art. The technique used for a given cell depends on the known successful techniques. Means for introducing recombinant DNA into cells include microinjection, transfection mediated by DEAE-dextran, transfection mediated by liposomes such as by using lipofectamine (Gibco, MD, USA) and/or cellfectin (Gibco, MD, USA), PEG-mediated DNA uptake, electroporation and microparticle bombardment such as by using DNA-coated tungsten or gold particles (Agracetus Inc., WI, USA) amongst others.

The host cells used to produce the protein may be cultured in a variety of media, depending on the cell type used. Commercially available media such as Ham's Fl0 (Sigma), Minimal Essential Medium ((MEM), (Sigma), RPMl-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing mammalian cells. Media for culturing other cell types discussed herein are known in the art.

Isolation of Proteins

Methods for isolating a protein are known in the art and/or described herein.

Where an IL-11Rα-binding protein is secreted into culture medium, supernatants from such expression systems can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants. Alternatively, or additionally, supernatants can be filtered and/or separated from cells expressing the protein, e.g., using continuous centrifugation.

The IL-11Rα-binding protein prepared from the cells can be purified using, for example, ion exchange, hydroxyapatite chromatography, hydrophobic interaction chromatography, gel electrophoresis, dialysis, affinity chromatography (e.g., protein A affinity chromatography or protein G chromatography), or any combination of the foregoing. These methods are known in the art and described, for example in WO99/57134 or Ed Harlow and David Lane (editors) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, (1988).

The skilled artisan will also be aware that a protein can be modified to include a tag to facilitate purification or detection, e.g., a poly-histidine tag, e.g., a hexa-histidine tag, or a influenza virus hemagglutinin (HA) tag, or a Simian Virus 5 (V5) tag, or a FLAG tag, or a glutathione S-transferase (GST) tag. The resulting protein is then purified using methods known in the art, such as, affinity purification. For example, a protein comprising a hexa-his tag is purified by contacting a sample comprising the protein with nickel-nitrilotriacetic acid (Ni-NTA) that specifically binds a hexa-his tag immobilized on a solid or semi-solid support, washing the sample to remove unbound protein, and subsequently eluting the bound protein. Alternatively, or in addition a ligand or antibody that binds to a tag is used in an affinity purification method.

Conjugates

In one example, an IL-11Rα-binding protein of the present disclosure is conjugated to a compound. For example, the compound is selected from the group consisting of a radioisotope, a detectable label, a therapeutic compound, a colloid, a toxin, a nucleic acid, a peptide, a protein, a compound that increases the half life of the IL-11Rα-binding protein in a subject and mixtures thereof.

The other compound can be directly or indirectly bound to the IL-11Rα-binding protein (e.g., can comprise a linker in the case of indirect binding). Examples of compounds include, a radioisotope (e.g., iodine-131, yttrium-90 or indium-111), a detectable label (e.g., a fluorophore or a fluorescent nanocrystal or quantum dot), a therapeutic compound (e.g., a chemotherapeutic or an anti-inflammatory), a colloid (e.g., gold), a toxin (e.g., ricin or tetanus toxoid), a nucleic acid, a peptide (e.g., a serum albumin binding peptide), a protein (e.g., a protein comprising an antigen binding domain of an antibody or serum albumin), a compound that increases the half life of the IL-11Rα-binding protein in a subject (e.g., polyethylene glycol or other water soluble polymer having this activity) and mixtures thereof. Exemplary compounds that can be conjugated to a IL-11Rα-binding protein of the disclosure and methods for such conjugation are known in the art and described, for example, in WO2010/059821.

The IL-11Rα-binding protein may be conjugated to nanoparticles (for example as reviewed in Kogan *et al., Nanomedicine (Lond). 2*: 287-306, 2007). The nanoparticles may be metallic nanoparticles.

The IL-11Rα-binding protein may be comprised in antibody-targeted bacterial minicells (for example as described in PCT/IB2005/000204).

Some exemplary compounds that can be conjugated to a IL-11Rα-binding protein of the present disclosure are listed in Table 2.

Table 2. Compounds useful in conjugation.

Group	Detail
Radioisotopes (either directly or indirectly)	• ^{123}I, ^{125}I, ^{130}I, ^{133}I, ^{135}I, ^{47}Sc, ^{72}As , ^{72}Sc, ^{90}Y, ^{88}Y, ^{97}Ru, ^{100}Pd, ^{101m}Rh, ^{101m}Rh, ^{119}Sb, ^{128}Ba, ^{197}Hg, ^{211}At, ^{212}Bi, ^{153}Sm, ^{169}Eu, ^{212}Pb, ^{109}Pd, ^{111}In , ^{67}Gu, ^{68}Gu, ^{67}Cu, ^{75}Br, ^{76}Br , ^{77}Br, ^{99m}Tc, ^{11}C, ^{13}N, ^{15}O, ^{18}I, ^{188}Rc, ^{203}Pb, ^{64}Cu, ^{105}Rh, ^{198}Au, ^{199}Ag or ^{177}Lu
Half life extenders	• Polyethylene glycol • Glycerol • Glucose
Fluorescent probes	• Phycoerythrin (PE) • Allophycocyanin (APC) • Alexa Fluor 488 • Cy5.5
Biologics	• fluorescent proteins such as Renilla luciferase, GFP • immune modulators or proteins, such as cytokines, e.g., an interferon • toxins • an immunoglobulin or antibody or antibody variable region • half life extenders such as albumin or antibody variable regions or peptides that bind to albumin
Chemotherapeutics	• Taxol • 5-FU • Doxorubicin • Idarubicin

Assaying Activity of an IL-11Rα-Binding Protein

Binding to IL-11Rα and Mutants Thereof

It will be apparent to the skilled artisan from the disclosure herein that some IL-11Rα-binding proteins of the present disclosure bind to the extracellular region (e.g., a region as described herein) of hIL-11Rα and to specific mutant forms of extracellular region of hIL-11Rα (e.g., SEQ ID NO: 3 or SEQ ID NO: 85 without or with certain point mutations) and/or bind to both human and cynomolgus monkey IL-11Rα.

Methods for assessing binding to a protein are known in the art, e.g., as described in Scopes (*In:* Protein purification: principles and practice, Third Edition, Springer Verlag, 1994). Such a method generally involves immobilizing the IL-11Rα-binding protein and contacting it with labeled antigen. Following washing to remove non-specific bound protein, the amount of label and, as a consequence, bound antigen is detected. Of course, the IL-11Rα-binding protein can be labeled and the antigen immobilized. Panning-type assays can also be used. Alternatively, or additionally, surface plasmon resonance assays can be used.

The assays described above can also be used to detect the level of binding of a protein to hIL-11Rα or an extracellular region thereof (e.g., as contained within SEQ ID NO: 3) or to a polypeptide of SEQ ID NO: 3 or SEQ ID NO: 85 or mutant form thereof.

In one example, an IL-11Rα-binding protein of the present disclosure binds to a polypeptide of SEQ ID NO: 95 at a level at least about 1.5 fold or 2 fold or 5 fold or 10 fold or 50 fold or 100 fold or 150 fold or 160 fold or 200 fold lower than it binds to a polypeptide of SEQ ID NO: 85.

In one example, a protein of the present disclosure binds to a polypeptide of SEQ ID NO: 96 at a level at least about 1.5 fold or 2 fold or 5 fold or 10 fold or 50 fold or 100 fold or 150 fold or 160 fold or 200 fold lower than it binds to a polypeptide of SEQ ID NO: 85.

In one example, a protein of the present disclosure binds to a polypeptide of SEQ ID NO: 86 at a level at least about 1.5 fold or 2 fold or 5 fold or 10 fold or 50 fold or 100 fold or 150 fold or 160 fold or 200 fold lower than it binds to a polypeptide of SEQ ID NO: 85.

In one example, a protein of the present disclosure binds to a polypeptide of SEQ ID NO: 89 at a level at least about 1.5 fold or 2 fold or 5 fold or 10 fold or 50 fold or 100 fold or 150 fold or 160 fold or 200 fold lower than it binds to a polypeptide of SEQ ID NO: 85.

The level of binding is conveniently determined using a biosensor.

The present disclosure contemplates any combination of the foregoing characteristics. In one example, a protein described herein has all of the binding characteristics set forth in the preceding five paragraphs.

Epitope Mapping

In another example, the epitope bound by a protein described herein is mapped. Epitope mapping methods will be apparent to the skilled artisan. For example, a series

of overlapping peptides spanning the IL-11Rα sequence or a region thereof comprising an epitope of interest, e.g., peptides comprising 10-15 amino acids are produced. The IL-11Rα-binding protein is then contacted to each peptide and the peptide(s) to which it binds determined. This permits determination of peptide(s) comprising the epitope to which the protein binds. If multiple non-contiguous peptides are bound by the protein, the protein may bind a conformational epitope.

Alternatively, or in addition, amino acid residues within IL-11Rα are mutated, e.g., by alanine scanning mutagenesis or substitution of evolutionarily conserved amino acids, and mutations that reduce or prevent binding of the IL-11Rα-binding protein are determined. Any mutation that reduces or prevents binding of the IL-11Rα-binding protein is likely to be within the epitope bound by the IL-11Rα-binding protein.

In this regard, as shown herein, mutation of the valine at position 117 of IL-11R relative to SEQ ID NO: 1 reduced or prevented binding of 8E2 and 8D10. Further testing of affinity matured variants of 8E2 confirmed that the V117 residue of IL-11R was more important for binding relative to the other residues that were analyzed by mutation.

Another method for determining a region comprising an epitope bound by an IL-11Rα-binding protein involved substituting a region of hIL-11Rα with the corresponding region of a form of IL-11Rα to which the IL-11Rα-binding protein does not bind (e.g., mIL-11Rα). If the IL-11Rα-binding protein does not bind to the mutant form of IL-11Rα, then residues forming a part of the epitope of the protein may be within the substituted region.

A further method for determining a region comprising an epitope involves binding IL-11Rα or a region thereof to an immobilized IL-11Rα-binding protein of the present disclosure and digesting the resulting complex with proteases. Peptide that remains bound to the immobilized IL-11Rα-binding protein are then isolated and analyzed, e.g., using mass spectrometry, to determine their sequence.

A further method involves converting hydrogens in IL-11Rα or a region thereof to deutrons and binding the resulting protein to an immobilized IL-11Rα-binding protein of the present disclosure. The deutrons are then converted back to hydrogen, the IL-11Rα or region thereof isolated, digested with enzymes and analyzed, e.g., using mass spectrometry to identify those regions comprising deutrons, which would have been protected from conversion to hydrogen by the binding of an IL-11Rα-binding protein described herein.

Optionally, the dissociation constant (Kd), association constant (Ka) and/or affinity constant (K_D) of an immobilized IL-11Rα-binding protein for IL-11Rα or an

epitope thereof is determined. The "Kd" or "Ka" or "K_D" for an IL-11Rα-binding protein is in one example measured by a radiolabeled or fluorescently-labeled IL-11Rα binding assay. In the case of a "Kd", this assay equilibrates the IL-11Rα-binding protein with a minimal concentration of labeled IL-11Rα in the presence of a titration series of unlabeled IL-11Rα. Following washing to remove unbound IL-11Rα, the amount of label is determined, which is indicative of the Kd of the protein.

According to another example the Kd, Ka or K_D is measured by using surface plasmon resonance assays, e.g., using BIAcore surface plasmon resonance (BIAcore, Inc., Piscataway, NJ) with immobilized IL-11Rα or a region thereof or immobilized IL-11Rα-binding protein.

In some examples, the IL-11Rα-binding protein has a similar K_D or an improved K_D (i.e., a K_D value lower than) than antibody 8E2, because they are likely to compete for binding to IL-11Rα.

Determining Competitive Binding

Assays for determining a protein that competitively inhibits binding of antibody 8E2 and/or 8D10 and/or 8E4 will be apparent to the skilled artisan. One such method is exemplified herein.

For example, the antibody is conjugated to a detectable label, e.g., a fluorescent label or a radioactive label. The labeled antibody and the test IL-11Rα-binding protein are then mixed and contacted with IL-11Rα or a region thereof (e.g., as contained within a polypeptide comprising SEQ ID NO: 3) or a cell expressing same. The level of labeled antibody is then determined and compared to the level determined when the labeled antibody is contacted with the IL-11Rα, region or cells in the absence of the IL-11Rα-binding protein. If the level of labeled antibody is reduced in the presence of the test IL-11Rα-binding protein compared to the absence of the IL-11Rα-binding protein, the IL-11Rα-binding protein is considered to competitively inhibit binding of the antibody to IL-11Rα.

Optionally, the test IL-11Rα-binding protein is conjugated to different label to the antibody. This alternate labeling permits detection of the level of binding of the test IL-11Rα-binding protein to IL-11Rα or the region thereof or the cell.

In another example, the IL-11Rα-binding protein is permitted to bind to IL-11Rα or a region thereof (e.g., as contained within a polypeptide comprising SEQ ID NO: 3) or a cell expressing same prior to contacting the IL-11Rα, region or cell with the antibody. A reduction in the amount of bound antibody in the presence of the IL-11Rα-binding protein compared to in the absence of the IL-11Rα-binding protein

indicates that the protein competitively inhibits binding of the antibody to IL-11Rα. A reciprocal assay can also be performed using labeled IL-11Rα-binding protein and first allowing the antibody to bind to IL-11Rα. In this case, a reduced amount of labeled IL-11Rα-binding protein bound to IL-11Rα in the presence of the antibody compared to in the absence of the antibody indicates that the IL-11Rα-binding protein competitively inhibits binding of the antibody to IL-11Rα.

Any of the foregoing assays can be performed with a mutant form of IL-11Rα and/or SEQ ID NO: 3 and/or SEQ ID NO: 85 and/or an extracellular region of IL-11Rα to which 8E2 and/or 8D10 and/or 8E4 binds, e.g., as described herein.

Determining Neutralization

In some examples of the present disclosure, a protein is capable of neutralizing IL-11 signaling.

Various assays are known in the art for assessing the ability of a protein to neutralize signaling of a ligand through a receptor.

In one example, the protein reduces or prevents IL-11 binding to IL-11Rα. These assays can be performed as a competitive binding assay as described herein using labeled IL-11 and/or labeled IL-11Rα-binding protein.

In a further example, the IL-11Rα-binding protein reduces proliferation of cells (e.g., BaF3 cells) expressing IL-11Rα and gp130 (e.g., cells modified to express the both proteins) which are cultured in the presence of IL-11. Cells (e.g., about $1x10^4$ cell) are cultured in the presence of IL-11 (e.g., between about 0.3ng/mL to about 5ng/mL (such as 0.3ng/mL or 0.5ng/mL or 5ng/mL for hIL-11 or 0.5ng/mL or 5ng/mL for cynoIL-11) or between about 1ng/mL to about 5ng/mL (such as 1ng/mL or 3 ng/mL or 5ng/mL) for mIL-11) and the presence or absence of a test IL-11Rα-binding protein. Methods for assessing cell proliferation are known in the art and include, for example, MTT reduction and/or thymidine incorporation. An IL-11Rα-binding protein that reduces the level of proliferation compared to the level observed in the absence of the IL-11Rα-binding protein is considered to neutralize IL-11R signaling. By testing multiple concentrations of the IL-11Rα-binding protein an IC_{50} is determined, i.e., a concentration at which 50% of the maximum inhibition of cell proliferation occurs. IC_{50} of 10μg/ml or less. In one example, the IC_{50} is 9μg/ml or less. For example, the IC_{50} is 8μg/ml or less. For example, the IC_{50} is 7μg/ml or less. For example, the IC_{50} is 6μg/ml or less. For example, the IC_{50} is 5μg/ml or less. For example, the IC_{50} is 4μg/ml or less. For example, the IC_{50} is 3μg/ml or less. In one example, relating to each of the foregoing examples, the IC_{50} can be 10pg/ml or more or 10ng/ml or more.

A similar assay to that described in the foregoing paragraph can be performed with B9 cells or T10 cells (Dams-Kozlowska *et al., BMC Biotechnol, 12*: 8, 2012; and Yokote *et al., J AOAC, 83*: 1053-1057, 2000). In the case of an assay making use of T10 cells, proliferation can be measured by colorimetrically detecting reduction of the tetrazolium compound, 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate (WST-1).

In a further example, the ability of the IL-11Rα-binding protein to suppress IL-11-mediated erythropoiesis is assessed. For example, Lin^-CD34^+ cells (e.g., from cord blood) are contacted with IL-11 in the presence or absence of the IL-11Rα-binding protein. The amount of erythropoiesis is determined by detecting the number of CD235a expressing cells, e.g., using FACS. An IL-11Rα-binding protein that reduces the number of CD235a expressing cells compared to the number in the absence of the IL-11Rα-binding protein is considered to neutralize IL-11 signaling.

In a still further example, the ability of the IL-11Rα-binding protein to suppress IL-11-mediated STAT3 phosporylation is assessed. For example, cells expressing IL-11Rα and gp130 are cultured in the presence of IL-11 in the presence or absence of the IL-11Rα-binding protein. The level of STAT3 phosphorylation is then assessed by Western blotting or FACS using an antibody specific for phosphorylated STAT3. An exemplary assay making use of FACS is described in Dams-Kozlowska *et al., BMC Biotechnol, 12*: 8, 2012.

In another example, the ability of the IL-11Rα-binding protein to suppress IL-11-mediated proliferation of cancer cells, e.g., gastric cancer cells or acute myelogenous leukemia (AML) cells is assessed. In these assays cancer cells (e.g., AGN or MKN45 gastric cancer cells) are cultured in the presence of IL-11 in the presence or absence of the IL-11Rα-binding protein. In the case of AML cells, the cells may also be cultured in the presence of G-CSF. Proliferation of the cells is then measured using standard techniques, e.g., as discussed above and/or by assessing formation of L-CFU in the case of AML cells. Exemplary assays adaptable to the present disclosure are included in Zhang *et al., Int J Biol Sci., 8*: 383-393, 2012 and Kimura *et al., Leukemia, 13*: 1018-1027, 1999.

Other methods for assessing neutralization of IL-11 signaling are contemplated by the present disclosure.

Determining Effector Function

As discussed herein, some IL-11Rα-binding proteins of the present disclosure have reduced effector function or have effector function (or enhanced effector function). Methods for assessing ADCC activity are known in the art.

In one example, the level of ADCC activity is assessed using a ^{51}Cr release assay, an europium release assay or a ^{35}S release assay. In each of these assays, cells expressing IL-11R are cultured with one or more of the recited compounds for a time and under conditions sufficient for the compound to be taken up by the cell. In the case of a ^{35}S release assay, cells expressing IL-11Rα can be cultured with ^{35}S-labeled methionine and/or cysteine for a time sufficient for the labeled amino acids to be incorporated into newly synthesized proteins. Cells are then cultured in the presence or absence of the IL-11Rα-binding protein and in the presence of immune effector cells, e.g., peripheral blood mononuclear cells (PBMC) and/or NK cells. The amount of ^{51}Cr, europium and/or ^{35}S in cell culture medium is then detected, and little or no change in the presence of the IL-11Rα-binding protein compared to in the absence of IL-11Rα-binding protein indicates that the protein has reduced effector function and an increased amount compared to in the absence of the IL-11Rα-binding protein (or increased compared to in the presence of IL-11Rα-binding protein comprising an IgG1 Fc region) indicating effector function or enhanced effector function. Exemplary publications disclosing assays for assessing the level of ADCC induced by a protein include Hellstrom, *et al. Proc. Natl Acad. Sci. USA 83*:7059-7063, 1986 and Bruggemann, *et al., J. Exp. Med. 166*:1351-1361, 1987.

Other assays for assessing the level of ADCC induced by a protein include ACTI™ nonradioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. CA, USA) or CytoTox 96® non-radioactive cytotoxicity assay (Promega, WI, USA).

C1q binding assays may also be carried out to confirm that the IL-11Rα-binding protein is able to bind C1q and may induce CDC. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro *et al, J. Immunol. Methods 202*: 163, 1996).

Determining Half Life

Some IL-11Rα-binding proteins encompassed by the present disclosure have an improved half-life, e.g., are modified to extend their half-life compared to IL-11Rα-binding proteins that are unmodified. Methods for determining an IL-11Rα-binding protein with an improved half-life will be apparent to the skilled person. For example, the ability of an IL-11Rα-binding protein to bind to a neonatal Fc receptor (FcRn) is

assessed. In this regard, increased binding affinity for FcRn increases the serum half-life of the IL-11Rα-binding protein (see for example, Kim *et al., Eur J Immunol., 24*:2429, 1994).

The half-life of an IL-11Rα-binding protein of the disclosure can also be measured by pharmacokinetic studies, e.g., according to the method described by Kim *et al*, *Eur J of Immunol 24*:542, 1994. According to this method radiolabeled IL-11Rα-binding protein is injected intravenously into mice and its plasma concentration is periodically measured as a function of time, for example at 3 minutes to 72 hours after the injection. The clearance curve thus obtained should be biphasic, that is, an alpha phase and beta phase. For the determination of the *in vivo* half-life of the protein, the clearance rate in beta-phase is calculated and compared with that of the wild type or unmodified protein.

Assessing Therapeutic Efficacy

Assays for assessing therapeutic efficacy are described hereinabove in relation to determining neutralization by an IL-11Rα-binding protein.

In another example, the efficacy of a protein to treat a condition is assessed using an *in vivo* assay.

For example, the IL-11Rα-binding protein is tested in an animal model of arthritis. Exemplary models include a SKG strain of mouse (Sakaguchi et al., Nature, 426: 454-460), rat type II collagen arthritis model, mouse type II collagen arthritis model or antigen induced arthritis models in several species (Bendele *J Musculoskel Neuron Interact; 1(4)*:377-385, 2001). In these assays, arthritis is induced and the ability of the IL-11Rα-binding protein to reduce one or more symptoms of arthritis, e.g., joint inflammation and/or markers of inflammation in synovial fluid is assessed. An IL-11Rα-binding protein that reduces a symptom of arthritis is considered useful for treating this condition or an IL-11-mediated condition (e.g., an IL-11-mediated inflammatory condition).

The IL-11Rα-binding protein can also or alternatively be tested in a model of COPD, e.g., in which a non-human mammal (e.g., a rodent, such as, a mouse) is exposed to cigarette smoke. Following exposure, the mammal is administered an IL-11Rα-binding protein and the level of lung inflammation and/or the number of neutrophils in the lung is assessed or estimated using standard techniques. An IL-11Rα-binding protein that reduces lung inflammation and/or the number of neutrophils is considered useful for treating lung inflammation or COPD or an IL-11-mediated

condition (e.g., an IL-11-mediated inflammatory condition, such as an IL-11-mediated inflammatory lung condition).

The IL-11Rα-binding protein can also or alternatively be tested in a Th2-inflammatory condition, such as asthma or airway hyperreactivity. An exemplary model of allergic asthma is the mouse OVA-model, e.g., as described in Wang *et al, J. Immunol. 165*: 2222, 2000. Following induction of inflammation, an IL-11Rα-binding protein is administered to the mice and symptoms of asthma, such as numbers of eosinophils in bronchoalveolar lavage fluid (BAL), mucus secretion and/or goblet cell hyperplasia are assessed. Other models of asthma are known in the art and include an ovine model of inflammatory asthma as described in WO2002/098216, a mouse model of allergic asthma, e.g., induced by host dust mite protein (Fattouh *et al., Am J Respir Crit Care Med 172*: 314–321, 2005), a mouse model of severe asthma in which IL-5 and eotaxin are overexpressed, or mice receiving intratracheal instillation of poly-l-lysine which are hypersensitive to methacholine when delivered as an aerosol (Homma *et al., Am J Physiol Lung Cell Mol Physiol 289*: L413–L418, 2005).

The IL-11Rα-binding protein can additionally or alternatively be tested in a model of cancer, e.g., gastric cancer. For example, mice homologous for the Y757F mutant of gp130 ($gp130^{Y757F/Y757F}$) develop gastric tumors Jenkins *et al, Blood 109*: 2380-2388, 2007). Mice (e.g., eight week old mice) are treated with an IL-11Rα-binding protein and the number and/or weight of gastric polyps assessed. An IL-11Rα-binding protein that reduces polyp size and/or weight is considered useful for treating cancer. A similar assay can be used to test for an effect on colon cancer, in which $gp130^{Y757F/Y757F}$ mice are treated with azoxymethane (AOM) followed by dextran sodium sulfate (DSS) essentially as described in Greten (*et al, Cell, 118*: 285-296, 2004) to induce colon cancer prior to treatment with the IL-11Rα-binding protein.

The IL-11Rα-binding protein can additionally or alternatively be tested in a model of cancer metastasis or cancer-related bone disease, e.g., as described in Li *et al., Oncol. Lett. 3*: 802-806, 2012.

Conditions to be Treated

The present disclosure contemplates treatment or prevention of any condition that is caused by or exacerbated by IL-11 in a subject.

In one example, the condition is an autoimmune or inflammatory condition.

In one example, the autoimmune condition is an autoimmune joint condition, such as, inflammatory arthritis, rheumatoid arthritis or idiopathic arthritis, e.g., juvenile idiopathic arthritis. In one example, the condition is rheumatoid arthritis.

In one example, the autoimmune condition is an autoimmune bowel condition, such as inflammatory bowel disease, such as ulcerative colitis or Crohn's disease.

In one example, the autoimmune condition is an autoimmune skin condition, such as psoriasis.

In one example, the inflammatory condition is an inflammatory lung condition, such as, a pulmonary disease associated with neutrophil infiltration. For example, the condition is asthma, chronic obstructive pulmonary disease (COPD), rhinitis or allergy. In one example, the condition is asthma.

Other exemplary inflammatory conditions include infection-induced inflammation (e.g., inflammation induced by *M. tuberculosis*), gastric inflammation (e.g., associated with gastric cancer), or inflammatory dermatis (e.g., atopic dermatitis).

In one example, the condition is a wasting condition, such as cachexia or sarcopenia. In one example, wasting condition is cachexia. For example, the cachexia is associated with or caused by a condition selected from rheumatoid arthritis, diabetes, cardiac disease, chronic kidney disease, chronic pulmonary inflammation, intestinal inflammation, inflammatory bowel disease, age, sepsis or AIDS. In one example, the cachexia is associated with or caused by cancer.

In one example, the condition is a bone condition, e.g., caused by insufficient bone formation and/or excessive bone catabolism. Exemplary bone conditions include osteoporosis (including post-menopausal osteoporosis), bone fracture, bone resorption/damage caused by cancer (e.g., metastastic bone cancer, myeloma or Paget's disease of bone) and bone resorption/damage caused by treatment of cancer (e.g., chemotherapy, hormone ablation or hormone inhibition).

In one example, the condition is cancer. Exemplary cancers include hematologic cancers, cancers of epithelial origin, gastric cancer, pancreatic cancer, liver cancer, osteosarcoma, endometrial cancer or ovarian cancer.

In one example, the subject is resistant to, does not adequately respond to, or is unsuitable for treatment with another compound used to treat the condition. For example, the subject suffering from an autoimmune or inflammatory condition is resistant to, does not adequately respond to, or is unsuitable for treatment with a corticosteroid and/or an immunosuppressant and/or cyclophosphamide and and/or methotrexate and/or an anti-TNF antibody or soluble TNF receptor and/or an anti-CD20 antibody and/or an anti-IL6 antibody and/or an anti-CD22 antibody.

Compositions

In some examples, an IL-11Rα-binding protein as described herein can be administered orally, parenterally, by inhalation spray, adsorption, absorption, topically, rectally, nasally, bucally, vaginally, intraventricularly, via an implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically-acceptable carriers, or by any other convenient dosage form. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intraperitoneal, intrathecal, intraventricular, intrasternal, and intracranial injection or infusion techniques.

Methods for preparing an IL-11Rα-binding protein into a suitable form for administration to a subject (e.g. a pharmaceutical composition) are known in the art and include, for example, methods as described in Remington's Pharmaceutical Sciences (18th ed., Mack Publishing Co., Easton, Pa., 1990) and U.S. Pharmacopeia: National Formulary (Mack Publishing Company, Easton, Pa., 1984).

The pharmaceutical compositions of this disclosure are particularly useful for parenteral administration, such as intravenous administration or administration into a body cavity or lumen of an organ or joint. The compositions for administration will commonly comprise a solution of an IL-11Rα-binding protein dissolved in a pharmaceutically acceptable carrier, for example an aqueous carrier. A variety of aqueous carriers can be used, e.g., buffered saline and the like. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of an IL-11Rα-binding proteins of the present disclosure in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the patient's needs. Exemplary carriers include water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Nonaqueous vehicles such as mixed oils and ethyl oleate may also be used. Liposomes may also be used as carriers. The vehicles may contain minor amounts of additives that enhance isotonicity and chemical stability, e.g., buffers and preservatives.

Upon formulation, an IL-11Rα-binding proteins of the present disclosure will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically/prophylactically effective. Formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but other pharmaceutically acceptable forms are also contemplated, e.g., tablets, pills,

capsules or other solids for oral administration, suppositories, pessaries, nasal solutions or sprays, aerosols, inhalants, liposomal forms and the like. Pharmaceutical "slow release" capsules or compositions may also be used. Slow release formulations are generally designed to give a constant drug level over an extended period and may be used to deliver an IL-11Rα-binding protein of the present disclosure.

WO2002/080967 describes compositions and methods for administering aerosolized compositions comprising antibodies for the treatment of, e.g., asthma, which are also suitable for administration of an IL-11Rα-binding protein of the present disclosure.

Combination Therapies

In one example, an IL-11Rα-binding protein of the present disclosure is administered in combination with another compound useful for treating a condition described herein, either as combined or additional treatment steps or as additional components of a therapeutic formulation.

For example, the other compound is an anti-inflammatory compound. Alternatively, or additionally, the other compound is an immunosuppressant. Alternatively, or additionally, the other compound is a corticosteroid, such as prednisone and/or prednisolone. Alternatively, or additionally, the other compound is methotrexate. Alternatively, or additionally, the other compound is cyclophosphamide. Alternatively, or additionally, the other compound is mycophenolate mofetil. Alternatively, or additionally, the other compound is an anti-CD20 antibody (e.g., rituximab or ofatumumab). Alternatively, or additionally, the other compound is an anti-CD22 antibody (e.g., epratuzumab). Alternatively, or additionally, the other compound is an anti-TNF antibody (e.g., infliximab or adalimumab or golimumab) or soluble TNF receptor (e.g., etanercept). Alternatively, or additionally, the other compound is a CTLA-4 antagonist (e.g., abatacept, CTLA4-Ig). Alternatively, or additionally, the other compound is an anti-IL-6 antibody. Alternatively, or additionally, the other compound is a BLys antagonist, such as an anti-BLys antibody (e.g., belimumab).

In another example, the other compound is a chemotherapy drug or other drug used for treating cancer.

In another example, the protein described herein is administered before or after radiotherapy for the treatment of cancer.

Dosages and Timing of Administration

Suitable dosages of an IL-11Rα-binding proteins of the present disclosure will vary depending on the specific an IL-11Rα-binding protein, the condition to be treated and/or the subject being treated. It is within the ability of a skilled physician to determine a suitable dosage, e.g., by commencing with a sub-optimal dosage and incrementally modifying the dosage to determine an optimal or useful dosage. Alternatively, to determine an appropriate dosage for treatment/prophylaxis, data from the cell culture assays or animal studies are used, wherein a suitable dose is within a range of circulating concentrations that include the ED_{50} of the active compound with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. A therapeutically/prophylactically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (i.e., the concentration or amount of the compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma maybe measured, for example, by high performance liquid chromatography.

In some examples, a method of the present disclosure comprises administering a prophylactically or therapeutically effective amount of a protein described herein.

The term "therapeutically effective amount" is the quantity which, when administered to a subject in need of treatment, improves the prognosis and/or state of the subject and/or that reduces or inhibits one or more symptoms of a clinical condition described herein to a level that is below that observed and accepted as clinically diagnostic or clinically characteristic of that condition. The amount to be administered to a subject will depend on the particular characteristics of the condition to be treated, the type and stage of condition being treated, the mode of administration, and the characteristics of the subject, such as general health, other diseases, age, sex, genotype, and body weight. A person skilled in the art will be able to determine appropriate dosages depending on these and other factors. Accordingly, this term is not to be construed to limit the present disclosure to a specific quantity, e.g., weight or amount of protein(s), rather the present disclosure encompasses any amount of the IL-11Rα-binding protein(s) sufficient to achieve the stated result in a subject.

As used herein, the term "prophylactically effective amount" shall be taken to mean a sufficient quantity of a protein to prevent or inhibit or delay the onset of one or more detectable symptoms of a clinical condition. The skilled artisan will be aware

that such an amount will vary depending on, for example, the specific IL-11Rα-binding protein(s) administered and/or the particular subject and/or the type or severity or level of condition and/or predisposition (genetic or otherwise) to the condition. Accordingly, this term is not to be construed to limit the present disclosure to a specific quantity, e.g., weight or amount of IL-11Rα-binding protein(s), rather the present disclosure encompasses any amount of the IL-11Rα-binding protein(s) sufficient to achieve the stated result in a subject.

For *in vivo* administration of the IL-11Rα-binding protein described herein, normal dosage amounts may vary from about 10ng/kg up to about 100mg/kg of an individual's body weight or more per day. For repeated administrations over several days or longer, depending on the severity of the disease or disorder to be treated, the treatment can be sustained until a desired suppression of symptoms is achieved.

In some examples, the IL-11Rα-binding protein is administered at an initial (or loading) dose of between about 1mg/kg to about 30mg/kg, such as from about 1mg/kg to about 10mg/kg, or about 1mg/kg or about 2mg/kg or 5mg/kg. The IL-11Rα-binding protein can then be administered at a lower maintenance dose of between about 0.01mg/kg to about 2mg/kg, such as from about 0.05mg/kg to about 1mg/kg, for example, from about 0.1mg/kg to about 1mg/kg, such as about 0.1mg/kg or 0.5mg/kg or 1mg/kg. The maintenance doses may be administered every 7-30 days, such as, every 10-15 days, for example, every 10 or 11 or 12 or 13 or 14 or 15 days.

In some examples, the IL-11Rα-binding protein is administered at a dose of between about 0.01mg/kg to about 50mg/kg, such as between about 0.05mg/kg to about 30mg/kg, for example, between about 0.1mg/kg to about 20mg/kg, for example, between about 0.1mg/kg to about 10mg/kg, such as between about 0.1mg/kg to about 2mg/kg. For example, the IL-11Rα-binding protein is administered at a dose of between about 0.01mg/kg to about 5mg/kg, such as from about 0.1mg/kg to about 2mg/kg, such as about 0.2mg/kg or 0.3mg/kg or 0.5mg/kg or 1mg/kg or 1.5mg/kg (e.g., without a higher loading dose or a lower maintenance dose). In some examples, numerous doses are administered, e.g., every 7-30 days, such as, every 10-22 days, for example, every 10-15 days, for example, every 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 days. For example, the IL-11Rα-binding protein is administered every 7 days or every 14 days or every 21 days.

In some examples, at the time of commencing therapy, the mammal is administered the IL-11Rα-binding protein on no more than 7 consecutive days or 6 consecutive days or 5 consecutive days or 4 consecutive days.

In the case of a mammal that is not adequately responding to treatment, multiple doses in a week may be administered. Alternatively, or in addition, increasing doses may be administered.

In another example, for mammals experiencing an adverse reaction, the initial (or loading) dose may be split over numerous days in one week or over numerous consecutive days.

Administration of an IL-11Rα-binding protein according to the methods of the present disclosure can be continuous or intermittent, depending, for example, on the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of an IL-11Rα-binding protein may be essentially continuous over a preselected period of time or may be in a series of spaced doses, e.g., either during or after development of a condition.

IL-11Rα Detection Assays

The following assays can be performed with an IL-11Rα-binding protein of the disclosure, e.g., an IL-11Rα-binding protein conjugated to a detectable label as discussed herein. Detection of IL-11Rα or cells expressing same with an assay described herein is useful for diagnosing or prognosing a condition.

An immunoassay is an exemplary assay format for diagnosing a condition in a subject or detecting IL-11Rα and cells expressing same in a sample. The present disclosure contemplates any form of immunoassay, including Western blotting, enzyme-linked immunosorbent assay (ELISA), fluorescence-linked immunosorbent assay (FLISA), competition assay, radioimmunoassay, lateral flow immunoassay, flow-through immunoassay, electrochemiluminescent assay, nephelometric-based assays, turbidometric-based assay, and fluorescence activated cell sorting (FACS)-based assays.

One form of a suitable immunoassay is, for example, an ELISA or FLISA.

In one form such an assay involves immobilizing an IL-11Rα-binding protein of the disclosure onto a solid matrix, such as, for example a polystyrene or polycarbonate microwell or dipstick, a membrane, or a glass support (e.g. a glass slide). A test sample is then brought into direct contact with the IL-11Rα-binding protein and IL-11Rα or cells expressing same in the sample is bound or captured. Following washing to remove any unbound protein in the sample, an IL-11Rα-binding protein that binds to IL-11Rα at a distinct epitope or binds to a different antigen on a cell is brought into direct contact with the captured IL-11Rα or cell. This detector protein is generally labeled with a detectable reporter molecule, such as for example, an enzyme (e.g.

horseradish peroxidase (HRP), alkaline phosphatase (AP) or β-galactosidase) in the case of an ELISA or a fluorophore in the case of a FLISA. Alternatively, a second labeled protein can be used that binds to the detector protein. Following washing to remove any unbound protein the detectable reporter molecule is detected by the addition of a substrate in the case of an ELISA, such as for example hydrogen peroxide, TMB, or toluidine, or 5-bromo-4-chloro-3-indol-beta-D-galaotopyranoside (x-gal). Of course, the immobilized (capture) protein and the detector protein may be used in the opposite manner.

The level of the antigen in the sample is then determined using a standard curve that has been produced using known quantities of the marker or by comparison to a control sample.

The assays described above are readily modified to use chemiluminescence or electrochemiluminescence as the basis for detection.

As will be apparent to the skilled artisan, other detection methods based on an immunosorbent assay are useful in the performance of the present disclosure. For example, an immunosorbent method based on the description *supra* using a radiolabel for detection, or a gold label (e.g. colloidal gold) for detection, or a liposome, for example, encapsulating NAD+ for detection or an acridinium linked immunosorbent assay.

In some examples of the disclosure, the level of IL-11Rα or cell expressing same is determined using a surface plasmon resonance detector (e.g., BIAcore™, GE Healthcare, Piscataway, N.J.), a flow through device, for example, as described in US7205159; a micro- or nano-immunoassay device (e.g., as described in US20030124619); a lateral flow devices (e.g., as described in US20040228761 or US20040265926); a fluorescence polarization immunoassay (FPIA e.g., as described in US4593089 or US4751190); or an immunoturbidimetric assay (e.g., as described in US5571728 or US6248597).

Samples and Control Samples

As will be apparent to the skilled artisan, some of the examples described herein require some degree of quantification to determine the level of IL-11Rα or cell expressing same. Such quantification may be determined by the inclusion of a suitable control sample in an assay of the disclosure.

In one example, a suitable control sample is a sample that is derived from a healthy subject or a normal subject.

In the present context, the term "healthy subject" shall be taken to mean an individual who is known not to suffer from a condition associated with IL-11Rα, e.g., an inflammatory condition.

The term "normal subject" shall be taken to mean an individual having a normal level of IL-11Rα or cell expressing same in a sample compared to a population of individuals.

The present disclosure also contemplates the control sample as being a data set obtained from a normal and/or healthy subject or a population of normal and/or healthy subjects.

In one example, a method of the disclosure additionally comprises determining the level of IL-11Rα in a control sample, e.g., using a method described herein.

In one example, a sample from the subject and a control sample are assayed at approximately or substantially the same time.

In one example, the sample from the subject and the control sample are assayed using the same method of the disclosure as described herein in any one or more examples to allow for comparison of results.

Kits

The present disclosure additionally comprises a kit comprising one or more of the following:

(i) an IL-11Rα-binding protein of the disclosure or expression construct(s) encoding same;

(ii) a cell of the disclosure; or

(iii) a pharmaceutical composition of the disclosure.

In the case of a kit for detecting IL-11Rα, the kit can additionally comprise a detection means, e.g., linked to a IL-11Rα-binding protein of the disclosure.

In the case of a kit for therapeutic/prophylactic use, the kit can additionally comprise a pharmaceutically acceptable carrier.

Optionally a kit of the disclosure is packaged with instructions for use in a method described herein according to any example.

The present disclosure includes the following non-limiting Examples.

Non-Limiting Examples

Materials and Methods

Phage display

Human IL-11Rα specific antibodies were isolated from a human Fab-phagemid antibody library. Phage that bound to immobilized hIL-11Rα-Fc (R&D Systems Cat. No. 1977-MR) were eluted in the presence of either wildtype hIL-11 or a hIL-11 mutein. A number of positive clones (as confirmed by phagemid ELISA) were reformatted to human IgG4 antibodies.

IL-11 responsive cell proliferation assay

hIL-11, cynoIL-11 and mIL-11 responsive BaF3 cell lines were used to test the ability of antibodies to block IL-11 bioactivity. The mIL-11 responsive BaF3 cell line is described in WO2009/052588.

hIL-11 and cynoIL-11 responsive BaF3 cell lines were stably transfected with constructs encoding wild-type human and cynoIL-11Rα, respectively, and human gp130 and cynomolgus monkey gp130, respectively. Cells were selected by growth in media containing hIL-11 or cynoIL-11 and clonal cell lines were derived by limit dilution cloning. A number of stably transfected clones were analyzed for their dose-responsive proliferation (using a thymidine incorporation assay) when cultured in the presence of hIL-11 or cyno IL-11. One hIL-11 responsive clone and one cynoIL-11 responsive clone were each selected for analysis of antibodies.

IL-11 responsive BaF3 cells were seeded at about 1 x 10^4 cells/well in 96 well plates in RPMI/10%FCS/Glutamax/PenStrep in the presence of a submaximal concentration of IL-11 (hIL-11: 0.3ng/mL or 0.5ng/mL or 5ng/mL; mIL-11: 1ng/mL, 3ng/mL or 5ng/mL; cyno IL-11: 0.5ng/mL or 5ng/mL) and increasing concentrations of purified monoclonal antibodies or a hIL-11 mutein (comprising SEQ ID NO: 110 and having a N-terminal hexa HIS tag) in a total volume of 200µL/well. Cells were initially cultured in the presence of antibody or hIL-11 mutein for 30 minutes prior to addition of cytokine. Cells were cultured for about 48-50 hours at 37°C and pulsed with ^{3}H-thymidine for the last 6 hours of culture. Cells were harvested onto glass fibre filters and the level of radioactive thymidine incorporated into DNA determined by liquid scintillation counting. Assays were performed in duplicate and mean values for each assay point were then plotted.

Affinity maturation of antibody 8E2

Antibody 8E2 was affinity matured using the following method. The sequences encoding the V_H and V_L of 8E2 were inserted into a phagemid construct to encode a germlined Fab and germline stop templates were created by replacing 18 codons (6 amino acid residues) in all CDRs, except CDR-L2, with TAA stop codons. Libraries were constructed using methods essentially as described by Sidhu *et al.* (*Methods in Enzymology*: *238*: 333-336, 2000). Each stop template was used as template for the Kunkel mutagenesis method (Kunkel *et al.*, *Methods in Enzymology: 154*: 367-382, 1987) with mutagenic oligonucleotides designed to simultaneously repair the stop codons and introduce mutations at the designed sites. The mutagenesis reactions were introduced into *E. coli* by electroporation, and phage production was initiated with addition of helper phage. After overnight growth at 30°C, the phage were harvested by precipitation with PEG/NaCl.

Libraries were cycled through several rounds of selection with decreasing concentration of biotinylated polypeptide comprising SEQ ID NO: 3. The target concentration was reduced 10-fold with each round.

Antibody binding to domain-swapped IL-11R mutant polypeptides

Various soluble forms of IL-11R comprising regions of hIL-11R and mIL-11R were produced and comprise sequences as set forth in SEQ ID NOs: 3 and 86-90 and the binding of antibodies 8E2, 8D10 and 8E4 to those polypeptides was determined using Western blotting or Biacore analysis.

For Biacore analysis, anti-human was IgG immobilized on the sensor surface at ~10,000-12,000RU and each antibody was captured at between 0.2µg/ml and 0.5µg/ml every cycle for 120 seconds. IL-11 receptors were injected over captured antibodies at varying concentrations, ranging from 1µM down to 2.5nM, including injections of buffer blanks. Association was monitored for 3-5 minutes, dissociation was monitored for 3-10 minutes. Approximate affinities were determined by fitting binding curves of each interaction to a 1:1 Kinetic Model.

Antibody binding to point mutants of soluble hIL-11R

Various point mutants of soluble forms of IL-11R were produced in which amino acids from mIL-11 were introduced into a soluble form of hIL-11 (SEQ ID NO: 85) (see Table 5 for positions of mutations relative to SEQ ID NO: 1 and for reference to the SEQ ID NO: of each polypeptide) and the binding of antibodies to those

polypeptides or to polypeptides of SEQ ID NO: 3 or SEQ ID NO: 85 was determined using Biacore analysis or FACS.

Anti-human IgG Fc specific antibody was chemically immobilized on a CM5 chip to ~9000 RU. Antibodies were captured at about 0.3-1 µg/ml for 120 seconds on 2 spots in each flow cell. Adjacent spots consisting of only anti human IgG were used as a reference. IL-11 receptors were injected over the captured antibodies and reference spots at 40,10, 5 and 2.5 nM in duplicate. Blank injections of buffer only were also performed in duplicate. Injection was performed for 3 minutes and dissociation monitored for a further 5 minutes.

Binding curves were reference subtracted and buffer blanked before fitting to a 1:1 kinetic model.

Antibody-mediated inhibition of IL-11 signaling in cancer cells

DLD-1 (colorectal cancer cell line) and MKN-28 (gastric cancer cell line) cells were stimulated with increasing concentrations of hIL-11 for 15 minutes or incubated in the presence of increasing concentrations of 8E2 anti-IL-11R or BM4 isotype control antibodies prior to stimulation with huIL-11 (50 ng/ml). Cells were fixed and permeabilized then stained with PE-conjugated anti-phosphoSTAT-3 antibodies. Cells were analyzed by flow cytometry. The assays were performed in duplicate.

Example 1: Antibody isolation and characterization

Three antibodies, 8E2, 8D10 and 8E4, were isolated from a Fab-phagemid antibody library based on their ability to inhibit hIL-11 dependent transfected BaF3 cell proliferation. 8E4 also inhibited mouse IL-11 dependent BaF3 cell proliferation.

8E2, 8D10 and 8E4 bound to cells transfected with hIL-11Rα or cynoIL-11Rα. 8E4, but not 8E2 and 8D10, bound to cells transfected with mIL-11Rα.

Of the three antibodies, 8E2 had the best thermal stability as assessed by differential scanning calorimetry (with a Tm of between 69.8°C and 76.6°C compared to between 63.2°C and 71.4°C for 8D10 and 8E4).

8E2 was selected and affinity matured. Heavy and light chain libraries were used to generate clones with mutated CDRs (Figures 1, 2 and 3). Binding of 62 of these affinity variants to hIL-11R was assessed by Biacore and relative ability to inhibit hIL-11 induced proliferation of BaF3 cells was determined. Results are shown in Table 3.

Table 3: Characteristics of affinity matured antibodies

mAb[1]	**IC_{50} (μg/ml)**[2]	**KD (M)**[3]	Fold increase in potency relative to 8E2
hu8E2-TS-82	0.26	2.05E-09	4.3
hu8E2-TS-79	0.21	2.13E-09	5.3
hu8E2-TS-88	0.36	2.25E-09	3.0
hu8E2-TS-71	0.29	2.49E-09	3.8
hu8E2-TS-76	0.32	2.73E-09	3.4
hu8E2-TS-92	0.18	3.20E-09	2.3
hu8E2-TS-69	1.46	3.56E-09	0.9
hu8E2-TS-89	0.12	4.50E-09	1.4
hu8E2-TS-91	0.34	4.73E-09	1.3
hu8E2-TS-66	2.84	7.46E-09	0.5
hu8E2-TS-115	0.26	7.35E-10	5.7
hu8E2-TS-101	0.19	7.85E-10	2.1
hu8E2-TS-104	0.28	9.95E-10	5.2
hu8E2-TS-97	0.26	1.22E-09	1.6
hu8E2-TS-107	0.20	1.29E-09	7.4
hu8E2-TS-108	0.12	1.93E-09	12.7
hu8E2-TS-151	0.26	2.42E-10	2.4
hu8E2-TS-136	0.16	2.32E-10	6.0
hu8E2-TS-143	0.39	4.16E-10	1.6
hu8E2-TS-140	0.42	4.71E-10	1.5
hu8E2-TS-133	0.23	5.13E-10	4.1
hu8E2-TS-134	0.22	5.20E-10	4.3
hu8E2-TS-135	0.20	5.25E-10	4.8
hu8E2-TS-129	0.20	6.30E-10	4.7
hu8E2-TS-156	0.13	6.31E-10	4.9
hu8E2-TS-221	0.20	6.34E-10	2.6
hu8E2-TS-214	0.31	1.11E-09	1.6
hu8E2-TS-218	0.19	1.39E-09	2.7
hu8E2-TS-215	0.34	1.85E-09	1.5
hu8E2-TS-224	0.39	1.91E-09	1.2
hu8E2-TS-222	0.16	1.91E-09	3.3

mAb[1]	**IC_{50} (μg/ml)**[2]	**KD (M)**[3]	Fold increase in potency relative to 8E2
hu8E2-TS-213*	0.51	> 0.00000000900	1.2
hu8E2-TS-306	0.15	1.07E-09	3.3
hu8E2-TS-307	0.25	1.32E-09	1.9
hu8E2-TS-305	0.23	1.58E-09	2.1
hu8E2-TS-311	0.60	1.69E-09	0.6
hu8E2-TS-312	0.29	1.76E-09	1.2
hu8E2-TS-310	0.84	2.09E-09	0.4
hu8E2-TS-303	0.35	4.09E-09	1.4
hu8E2-TS-313	0.51	5.56E-09	0.7
hu8E2-TS-322	0.33	6.01E-09	1.1
hu8E2-TS-7	0.63	1.08E-10	3.3
hu8E2-TS-20	1.30	2.84E-10	0.6
hu8E2-TS-6	0.41	2.89E-10	1.4
hu8E2-TS-4	0.31	2.95E-10	6.6
hu8E2-TS-14	0.52	3.42E-10	1.6
hu8E2-TS-21	0.31	2.30E-10	2.7
hu8E2-TS-17	0.35	3.90E-10	2.3
hu8E2-TS-103	0.33	4.97E-10	4.5
hu8E2-TS-22	0.40	8.38E-10	2.0
hu8E2-TS-2	0.26	5.29E-10	7.9
hu8E2-TS-29	0.33	1.06E-09	2.5
hu8E2-TS-9	0.68	2.29E-09	3.1
hu8E2-TS-32	0.41	9.56E-09	2.4
hu8E2-TS-13	NP		NP
hu8E2-TS-51	0.28	4.55E-10	3.6
hu8E2-TS-55	0.21	4.83E-10	4.8
hu8E2-TS-64	0.26	6.36E-10	5.4
hu8E2-TS-63	0.27	6.42E-10	5.1
hu8E2-TS-57	0.25	6.58E-10	3.9
hu8E2-TS-58	0.23	9.57E-10	6.0
hu8E2-TS-49	0.19	1.50E-09	5.2

mAb[1]	**IC_{50} (μg/ml)**[2]	**KD (M)**[3]	Fold increase in potency relative to 8E2
hu8E2 WT		4.54E-09	
hu8E2 WT 2		5.03E-09	
Mean hu8E2 WT (n=2)	N/D	4.78E-09	

[1] Sequence of antibody V_H and V_L as set out in Table 1

[2] Potency ((μg/ml) as determined in BaF Human IL-11R cell proliferation assay

[3] Biacore data generated at pH 8.5 and 37°C

NP means no potency; N/D means not determined

* some heterogeneity observed, an accurate K_D difficult to determine

Ten affinity matured antibodies, (TS-306, TS-2, TS-4, TS-7, TS-14, TS-51, TS-101, TS-108, TS-134 and TS-136,) were selected based on their improved ability to inhibit hIL-11 induced proliferation in BaF3 cells in comparison to 8E2, 8D10 and 8E4, and their improved affinity to human IL-11Rα compared to 8E2. CDR sequence was also considered in selecting these affinity matured antibodies. Some clones which had a CDR sequence close to or the same as the relevant consensus sequence were selected.

These antibodies were tested for inhibition of human, cyno and/or mouse IL-11-induced proliferation of BaF3 cells transfected with human, cyno or mouse IL-11R. Binding to human and cyno IL-11R transfected cells and measurement of mean fluorescence intensity by flow cytometry was also performed. Compared to the parental 8E2 clone, each of the selected clones more potently inhibited hIL-11 and cyno-IL-11-induced proliferation and bound with greater affinity to human and cyno IL-11R.

Example 2: Comparison of antagonism by hIL-11 mutein and antibodies

The antagonistic activity of several antibodies was compared to that of a hIL-11 mutein (comprising SEQ ID NO:110 and having a N-terminal hexa HIS tag)) using the assay described above using the BaF3 cell line transfected with nucleic acid encoding human IL-11R and human gp130 and 0.3ng/mL human IL-11. Results are presented in Table 4.

Table 4: Comparison of IL-11 signaling antagonism between anti-IL11R antibodies and a hIL-11 mutein

		average IC50 nM	**SD nM**
8E2		2.24	0.73
8E4		5.84	5.61
8D10		0.43	0.46
8E2-TS-51		1.14	0.13
8E2-TS-2		0.65	0.24
8E2-TS-134		2.50	0.63
8E2-TS-7		4.00	2.62
8E2-TS-4		1.22	0.32
8E2-TS-136		2.19	1.36
6xHis hIL-11 mutein		19.39	14.51

The data presented in Table 4 indicate that all antibodies tested inhibit IL-11 signaling more effectively than the hIL-11 mutein. Due to the large difference in molecular weight between the mutein and the antibodies, IC_{50} values are expressed in nM.

Example 3: Epitope Mapping

Competition ELISA and Biacore data indicate that 8E2, 8E4 and 8D10 compete with each other for binding to hIL-11Rα and may recognize the same region of hIL-11Rα.

Domain swapping (murine-human) Biacore data showed that at least amino acids 111-215 (Fn type 3 domain 1) of hIL-11Rα (SEQ ID NO: 1) was required for 8E2 and 8D10 binding and substituting mouse for human sequence in this region reduced the affinity of 8E4 binding (Table 5).

Table 5. Binding of IL-11 receptors to antibodies.

Sequence	8E4	8E2	8D10	4E5
SEQ ID NO: 86	weak binding	N/B	N/B	strong binding
SEQ ID NO: 87	strong binding	strong binding	strong binding	strong binding
SEQ ID NO: 88	strong binding	strong binding	strong binding	weak binding
SEQ ID NO: 89	weak binding	N/B	N/B	weak binding
SEQ ID NO: 3	N/A	strong binding	strong binding	weak binding
SEQ ID NO: 90	N/A	N/B	N/B	strong binding

N/A: not assessed

N/B: no or ablated binding

4E5 is a mouse monoclonal antibody with strong binding affinity to mouse IL-11Rα.

In the results presented in Table 5, reference to "strong binding" indicates affinities (K_D) of about 10nM or lower. Reference to "weak binding" indicates affinities (K_D) of about 50nM or higher.

Twenty eight constructs having a single amino acid substitution in the region covering both the Ig-like domain and the Fibronectin type 3 domain 1 of hIL-11Rα were made by replacing the native human amino acid with the corresponding mouse amino acid residue. Five mutants of a truncated hIL-11R (SEQ ID NO:85) (P65S, K66R, L101S, V117E, A178S) were expressed and purified (numbering relative to the amino acid position in SEQ ID NO:1). Corresponding constructs were used to transfect cells, which were subsequently stained with 8E2, 8E4 and 8D10. 8E2 and 8E10 binding was shown to be reduced in V117E transfected cells by flow cytometry.

Flow data were confirmed by Biacore. All Biacore sensorgrams fitted well to a 1:1 binding model and the derived K_D values are given in Table 6.

The V117E mutant did not bind to 8E2 and 8D10 at the concentrations used in this assay, and a K_D for this interaction could not be determined. This residue contributes to the binding interaction of these two antibodies.

The K66R mutation led to a decrease in K_D of just over twofold when compared to WT D1/2 (SEQ ID NO: 85) for antibodies 8E2 and 8D10, indicating some involvement of this residue in antibody binding.

The mutants V117E and K66R also bound to antibody 8E4 with a significantly lower affinity than WT D1/2 (SEQ ID NO: 85). These residues may contribute to the binding interaction of antibody 8E4 with hIL-11Rα.

ANOVA and Tukey's Multiple Comparisons tests indicated that both V95E and K44R affinities were significantly different ($p<0.05$) to WT D1/2 (SEQ ID NO: 85) for all antibodies tested.

All of the mutant forms of IL-11R tested in this example were able to bind to IL-11, indicating that the mutations did not induce a substantial conformational change in the receptor.

Table 6: Affinities of antibodies for point mutants of soluble human IL-11Rα (SEQ ID NO: 85)

Receptor / Mutant	**KD (nM)**		
	8E2	8E4	8D10
K66R (SEQ ID NO: 96)	**2.4 ± 0.13**	**5.6 ± 0.1**	**1.3 ± 0.09**
L101S (SEQ ID NO: 98)	1.17 ± 0.03	2.98 ± 0.06	0.7 ± 0.03
P65S (SEQ ID NO: 97)	1.08 ± 0.02	2.71 ± 0.1	0.67 ± 0.02
A178S (SEQ ID NO: 99)	1.1 ± 0.05	2.63 ± 0.1	0.6 ± 0.03
V117E (SEQ ID NO: 95)	*	**5.54 ± 0.1**	*
SEQ ID NO: 85	1.0, 1.1	3.2, 3.7	0.57, 0.69
SEQ ID NO: 85	1.0 ± 0.04	2.78 ± 0.08	0.58 ± 0.03
SEQ ID NO: 3	1.8 ± 0.06	4.0 ± 0.1	1.0 ± 0.03

Affinities of antibodies for various receptor mutants. N=2 for WT D1/2 (SEQ ID NO: 85), both values are shown. All other values are mean ± SE. N=3 for WT F/L (SEQ ID NO: 3), N=4 for all others. Values highlighted in bold have K_D values significantly different to WT D1/2 ($p<0.05$). Positions of mutations are relative to SEQ ID NO: 1. * indicates undetectable binding.

Example 4: Comparative data

A non-neutralizing anti-IL-11R antibody (4D12; commercially available antibody from Santa Cruz) was shown to bind to human IL-11Rα by ELISA. 4D12 was shown to bind to BaF3 cells transfected with human IL-11Rα and was shown to bind to 293 cells transfected with human or mouse IL-11Rα.

4D12 did not neutralize human or mouse IL-11 induced cellular proliferation in BaF3 cells incubated with 0.5ng/mL hIL-11 or 3ng/mL mIL-11R.

Competition ELISAs were uses to show that 4D12 does not compete for binding to IL-11Rα with either 8D10 or 8E2.

Example 5: Anti-IL-11R antibodies inhibit IL-11 signaling in cancer cells

As shown in Figure 4, IL-11 induces phosphorylation of STAT-3 in DLD-1 colon cancer cells and MKN-28 stomach cancer cells. Figure 4 also shows that antibody 8E2 inhibits STAT-3 phosphorylation in these cells, whereas an isotype control antibody does not.

CLAIMS:

1. An IL-11Rα-binding protein comprising an antigen binding domain of an antibody, wherein the antigen binding domain binds to or specifically binds to IL-11Rα and neutralizes IL-11 signaling, and wherein the antigen binding domain is capable of binding to hIL-11Rα and cynoIL-11Rα.

2. An IL-11Rα-binding protein comprising an antigen binding domain of an antibody, wherein the antigen binding domain binds to or specifically binds to IL-11Rα and neutralizes IL-11 signaling and the protein inhibits IL-11-mediated proliferation of BaF3 cells expressing IL-11Rα and gp130 with an IC_{50} of 10μg/ml or less.

3. An IL-11Rα-binding protein comprising an antigen binding domain of an antibody, wherein the antigen binding domain binds to or specifically binds to IL-11Rα and neutralizes IL-11 signaling and the level of binding of the IL-11Rα-binding protein to a polypeptide of SEQ ID NO: 86 or 89 is lower than the level of binding of the IL-11Rα-binding to a polypeptide of SEQ ID NO: 3 and/or 85.

4. The IL-11Rα-binding protein of claim 3, wherein the protein does not detectably bind to a polypeptide of SEQ ID NO: 86 or 89.

5. An IL-11Rα-binding protein comprising an antigen binding domain of an antibody, wherein the antigen binding domain binds to or specifically binds to IL-11Rα and neutralizes IL-11 signaling and the antigen binding domain binds to an epitope comprising residues within the first fibronectin III domain of IL-11Rα.

6. The IL-11Rα-binding protein of claim 5, wherein the epitope comprises residues within the immunoglobulin-like domain and the first fibronectin III domain of IL-11Rα

7. An IL-11Rα-binding protein comprising an antigen binding domain of an antibody, wherein the antigen binding domain binds to or specifically binds to IL-11Rα and neutralizes IL-11 signaling and wherein the protein competitively inhibits binding of antibody 8E2 (comprising a heavy chain comprising a sequence set forth in SEQ ID NO: 83 and a light chain comprising a sequence set forth in SEQ ID NO: 84) and/or antibody 8E4 (comprising a heavy chain comprising a sequence set forth in SEQ ID NO: 92 and a light chain comprising a sequence set forth in SEQ ID NO: 91) and/or antibody 8D10 (comprising a heavy chain comprising a sequence set forth in SEQ ID

NO: 94 and a light chain comprising a sequence set forth in SEQ ID NO: 93) to a polypeptide of SEQ ID NO: 3 and/or 85.

8. An IL-11Rα-binding protein comprising an antigen binding domain of an antibody, wherein the antigen binding domain binds to or specifically binds to IL-11Rα and neutralizes IL-11 signaling and wherein the level of binding of the IL-11Rα-binding protein to a polypeptide of SEQ ID NO: 95 is lower than the level of binding of the IL-11Rα-binding protein to a polypeptide of SEQ ID NO: 85.

9. The IL-11Rα-binding protein of claim 8, which competitively inhibits binding of antibody 8E2 (comprising a heavy chain comprising a sequence set forth in SEQ ID NO: 83 and a light chain comprising a sequence set forth in SEQ ID NO: 84) and/or 8E4 (comprising a heavy chain comprising a sequence set forth in SEQ ID NO: 92 and a light chain comprising a sequence set forth in SEQ ID NO: 91) and/or 8D10 ((comprising a heavy chain comprising a sequence set forth in SEQ ID NO: 94 and a light chain comprising a sequence set forth in SEQ ID NO: 93) to a polypeptide of SEQ ID NO: 3 and/or 85.

10. The IL-11Rα-binding protein of claim 8 or 9, wherein the antigen binding domain cross-reacts with:

(i) a polypeptide of SEQ ID NO: 97; and/or

(ii) a polypeptide of SEQ ID NO: 98; and/or

(iii) a polypeptide of SEQ ID NO: 99.

11. An IL-11Rα-binding protein comprising an antigen binding domain of an antibody, wherein the antigen binding domain binds to or specifically binds to IL-11Rα and neutralizes IL-11 signaling and wherein the antigen binding domain comprises at least one of:

(i) a V_H comprising a complementarity determining region (CDR) 1 comprising a sequence at least about 40% identical to a sequence set forth between amino acids 31-35 of SEQ ID NO: 37, a CDR2 comprising a sequence at least about 76% identical to a sequence set forth between amino acids 50-66 of SEQ ID NO: 37 and a CDR3 comprising a sequence at least about 55% identical to a sequence set forth between amino acids 99-107 of SEQ ID NO: 37;

(ii) a V_H comprising a sequence at least about 95% or 96% or 97% or 98% or 99% identical to a sequence set forth in SEQ ID NO: 37;

(iii) a V_L comprising a CDR1 comprising a sequence at least about 45% identical to a sequence set forth between amino acids 24-34 of SEQ ID NO: 5, a CDR2 comprising a sequence set forth between amino acids 50-56 of SEQ ID NO: 5 and a CDR3 comprising a sequence at least about 44% identical to a sequence set forth between amino acids 89-97 of SEQ ID NO: 5;

(iv) a V_L comprising a sequence at least about 94% identical to a sequence set forth in SEQ ID NO: 5;

(v) a V_H comprising a CDR1 comprising a sequence set forth between amino acids 31-35 of SEQ ID NO: 74, a CDR2 comprising a sequence set forth between amino acids 50-66 of SEQ ID NO: 74 and a CDR3 comprising a sequence set forth between amino acids 99-115 of SEQ ID NO: 74;

(vi) a V_H comprising a sequence set forth in SEQ ID NO: 74;

(vii) a V_L comprising a CDR1 comprising a sequence set forth between amino acids 23-36 of SEQ ID NO: 73, a CDR2 comprising a sequence set forth between amino acids 52-58 of SEQ ID NO: 73 and a CDR3 comprising a sequence set forth between amino acids 91-101 of SEQ ID NO: 73;

(viii) a V_L comprising a sequence set forth in SEQ ID NO: 73;

(ix) a V_H comprising a CDR1 comprising a sequence set forth between amino acids 31-35 of SEQ ID NO: 76, a CDR2 comprising a sequence set forth between amino acids 50-66 of SEQ ID NO: 76 and a CDR3 comprising a sequence set forth between amino acids 99-107 of SEQ ID NO: 76;

(x) a V_H comprising a sequence set forth in SEQ ID NO: 76;

(xi) a V_L comprising a CDR1 comprising a sequence set forth between amino acids 24-34 of SEQ ID NO: 75, a CDR2 comprising a sequence set forth between amino acids 50-57 of SEQ ID NO: 75 and a CDR3 comprising a sequence set forth between amino acids 89-97 of SEQ ID NO: 75;

(xii) a V_L comprising a sequence set forth in SEQ ID NO: 75;

(xiii) a V_H as set forth in (i) and a V_L as set forth in (iii);

(xiv) a V_H as set forth in (i) and a V_L as set forth in (iv);

(xv) a V_H as set forth in (ii) and a V_L as set forth in (iii);

(xvi) a V_H as set forth in (ii) and a V_L as set forth in (iv);

(xvii) a V_H as set forth in (v) and a V_L as set forth in (vii);

(xviii) a V_H as set forth in (v) and a V_L as set forth in (viii);

(xix) a V_H as set forth in (vi) and a V_L as set forth in (vii);

(xx) a V_H as set forth in (vi) and a V_L as set forth in (viii);

(xxi) a V_H as set forth in (ix) and a V_L as set forth in (xi);

(xxii) a V_H as set forth in (ix) and a V_L as set forth in (xii);
(xxiii) a V_H as set forth in (x) and a V_L as set forth in (xi); or
(xxiv) a V_H as set forth in (x) and a V_L as set forth in (xii).

12. The IL-11Rα-binding protein of claim 112, wherein the antigen binding domain comprises:
(i) a V_H comprising a sequence set forth in SEQ ID NO: 71 and a V_L comprising a sequence set forth in SEQ ID NO: 35; or
(ii) a V_H comprising a sequence set forth in SEQ ID NO: 72 and a V_L comprising a sequence set forth in SEQ ID NO: 36.

13. An IL-11Rα-binding protein comprising an antigen binding domain of an antibody, wherein the antigen binding domain binds to or specifically binds to IL-11Rα and neutralizes IL-11 signaling and wherein the antigen binding domain comprises:
(i) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;
(ii) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 5;
(iii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 38 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;
(iv) a V_H comprising a sequence set forth in SEQ ID NO: 38 and a V_L comprising a sequence set forth in SEQ ID NO: 5;
(v) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 39 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;
(vi) a V_H comprising a sequence set forth in SEQ ID NO: 39 and a V_L comprising a sequence set forth in SEQ ID NO: 5;
(vii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 40 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;
(viii) a V_H comprising a sequence set forth in SEQ ID NO: 40 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(ix) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 41 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(x) a V_H comprising a sequence set forth in SEQ ID NO: 41 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(xi) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 42 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(xii) a V_H comprising a sequence set forth in SEQ ID NO: 42 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(xiii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 43 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(xiv) a V_H comprising a sequence set forth in SEQ ID NO: 43 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(xv) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 44 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(xvi) a V_H comprising a sequence set forth in SEQ ID NO: 44 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(xvii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 45 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(xviii) a V_H comprising a sequence set forth in SEQ ID NO: 45 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(xix) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 46 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(xx) a V_H comprising a sequence set forth in SEQ ID NO: 46 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(xxi) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 47 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(xxii) a V_H comprising a sequence set forth in SEQ ID NO: 47 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(xxiii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 48 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(xxiv) a V_H comprising a sequence set forth in SEQ ID NO: 48 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(xxv) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 49 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(xxvi) a V_H comprising a sequence set forth in SEQ ID NO: 49 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(xxvii)a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 50 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(xxviii) a V_H comprising a sequence set forth in SEQ ID NO: 50 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(xxix) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 51 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(xxx) a V_H comprising a sequence set forth in SEQ ID NO: 51 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(xxxi) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 52 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(xxxii)a V_H comprising a sequence set forth in SEQ ID NO: 52 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(xxxiii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 53 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(xxxiv) a V_H comprising a sequence set forth in SEQ ID NO: 53 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(xxxv)a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 54 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(xxxvi) a V_H comprising a sequence set forth in SEQ ID NO: 54 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(xxxvii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 55 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(xxxviii) a V_H comprising a sequence set forth in SEQ ID NO: 55 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(xxxix) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 56 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(xl) a V_H comprising a sequence set forth in SEQ ID NO: 56 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(xli) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 57 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(xlii) a V_H comprising a sequence set forth in SEQ ID NO: 57 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(xliii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 58 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(xliv) a V_H comprising a sequence set forth in SEQ ID NO: 58 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(xlv) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 59 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(xlvi) a V_H comprising a sequence set forth in SEQ ID NO: 59 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(xlvii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 60 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(xlviii)a V_H comprising a sequence set forth in SEQ ID NO: 60 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(xlix) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 61 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(l) a V_H comprising a sequence set forth in SEQ ID NO: 61 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(li) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 62 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(lii) a V_H comprising a sequence set forth in SEQ ID NO: 62 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(liii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 63 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(liv) a V_H comprising a sequence set forth in SEQ ID NO: 63 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(lv) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 64 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(lvi) a V_H comprising a sequence set forth in SEQ ID NO: 64 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(lvii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 65 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(lviii) a V_H comprising a sequence set forth in SEQ ID NO: 65 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(lix) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 66 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(lx) a V_H comprising a sequence set forth in SEQ ID NO: 66 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(lxi) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 67 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(lxii) a V_H comprising a sequence set forth in SEQ ID NO: 67 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(lxiii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 68 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(lxiv) a V_H comprising a sequence set forth in SEQ ID NO: 68 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(lxv) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 69 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(lxvi) a V_H comprising a sequence set forth in SEQ ID NO: 69 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(lxvii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 70 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(lxviii)a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(lxix) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 70 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(lxx) a V_H comprising a sequence set forth in SEQ ID NO: 70 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(lxxi) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 6;

(lxxii) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 6;

(lxxiii)a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 7;

(lxxiv)a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 7;

(lxxv) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 8;

(lxxvi)a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 8;

(lxxvii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 9;

(lxxviii) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 9;

(lxxix)a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 10;

(lxxx) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 10;

(lxxxi)a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 11;

(lxxxii) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 11;

(lxxxiii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 12;

(lxxxiv) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 12;

(lxxxv) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 13;

(lxxxvi) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 13;

(lxxxvii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 14;

(lxxxviii)a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 14;

(lxxxix) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 15;

(xc) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 15;

(xci) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 16;

(xcii) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 16;

(xciii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 17;

(xciv) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 17;

(xcv) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 18;

(xcvi) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 18;

(xcvii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 19;

(xcviii) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 19;

(xcix) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 20;

(c) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 20;

(ci) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 21;

(cii) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 21;

(ciii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 22;

(civ) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 22;

(cv) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 23;

(cvi) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 23;

(cvii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 24;

(cviii) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 24;

(cix) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 25;

(cx) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 25;

(cxi) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 26;

(cxii) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 26;

(cxiii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 27;

(cxiv) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 27;

(cxv) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 28;

(cxvi) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 28;

(cxvii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 29;

(cxviii) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 29;

(cxix) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 30;

(cxx) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 30;

(cxxi) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 31;

(cxxii) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 31;

(cxxiii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 32;

(cxxiv) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 32;

(cxxv) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 33;

(cxxvi) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 33;

(cxxvii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 34; or

(cxxviii) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 34.

14. An IL-11Rα-binding protein comprising an antigen binding domain of an antibody, wherein the antigen binding domain binds to or specifically binds to IL-11Rα and neutralizes IL-11 signaling and wherein the antigen binding domain comprises:

(i) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 49 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(ii) a V_H comprising a sequence set forth in SEQ ID NO: 49 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(iii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 53 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(iv) a V_H comprising a sequence set forth in SEQ ID NO: 53 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(v) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 57 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(vi) a V_H comprising a sequence set forth in SEQ ID NO: 57 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(vii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 58 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(viii) a V_H comprising a sequence set forth in SEQ ID NO: 58 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(ix) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 8;

(x) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 8;

(xi) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 15;

(xii) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 15;

(xiii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 16;

(xiv) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 16;

(xv) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 18;

(xvi) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 18;

(xvii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 29; or

(xviii) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 29.

15. The IL-11Rα-binding protein of any one of claims 1 to 14 comprising at least a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein the V_H and V_L bind to form a Fv comprising an antigen binding domain, wherein the V_H and the V_L are in a single polypeptide chain or the V_L and V_H are in separate polypeptide chains.

16. The IL-11Rα-binding protein of claim 15, wherein if the V_H and V_L are in a single polypeptide chain, the protein is:

(i) a single chain Fv fragment (scFv);

(ii) a dimeric scFv (di-scFv);

(iii) one of (i) or (ii) linked to a constant region of an antibody, Fc or a heavy chain constant domain (C_H) 2 and/or C_H3; or

(iv) one of (i) or (ii) linked to a protein that binds to an immune effector cell, or

if the V_H and V_L are in separate polypeptide chains the protein is:

(i) a diabody;

(ii) a triabody;

(iii) a tetrabody;

(iv) a Fab;

(v) a $F(ab')_2$;

(vi) a Fv;

(vii) one of (i) to (vi) linked to a constant region of an antibody, Fc or a heavy chain constant domain (C_H) 2 and/or C_H3;

(viii) one of (i) to (vi) linked to a protein that binds to an immune effector cell; or

(ix) an antibody.

17. An antibody that binds to IL-11Rα and neutralizes IL-11 signaling, the antibody comprising:

(i) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 49 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(ii) a V_H comprising a sequence set forth in SEQ ID NO: 49 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(iii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 53 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(iv) a V_H comprising a sequence set forth in SEQ ID NO: 53 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(v) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 57 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(vi) a V_H comprising a sequence set forth in SEQ ID NO: 57 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(vii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 58 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 5;

(viii) a V_H comprising a sequence set forth in SEQ ID NO: 58 and a V_L comprising a sequence set forth in SEQ ID NO: 5;

(ix) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 8;

(x) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 8;

(xi) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 15;

(xii) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 15;

(xiii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 16;

(xiv) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 16;

(xv) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 18;

(xvi) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 18;

(xvii) a V_H comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 37 (optionally, also comprising the amino acid N-terminal to CDR1) and a V_L comprising CDRs 1, 2 and 3 of a sequence set forth in SEQ ID NO: 29; or

(xviii) a V_H comprising a sequence set forth in SEQ ID NO: 37 and a V_L comprising a sequence set forth in SEQ ID NO: 29.

18. The IL-11Rα-binding protein of any one of claims 1 to 16 or the antibody of claim 17, which is conjugated to another compound.

19. A nucleic acid encoding the IL-11Rα-binding protein of any one of claims 1 to 16 or the antibody of claim 17 or a polypeptide thereof.

20. An expression construct comprising the nucleic acid of claim 19.

21. An isolated or recombinant cell expressing the IL-11Rα-binding protein of any one of claims 1 to 16 or the antibody of claim 17.

22 A composition comprising an IL-11Rα-binding protein of any one of claims 1 to 16 or 18 or the antibody of claim 17 or 18 and a pharmaceutically acceptable carrier.

23. A method for treating or preventing an IL-11-mediated condition in a subject, the method comprising administering the IL-11Rα-binding protein of any one of claims 1 to 16 or 18 or the antibody of claim 17 or 18 or the composition of claim 22.

24. A method for preventing pregnancy in a subject, the method comprising administering the IL-11Rα-binding protein of any one of claims 1 to 16 or 18 or the antibody of claim 17 or 18 or the composition of claim 22.

25. Use of the IL-11Rα-binding protein of any one of claims 1 to 16 or 18 or the antibody of claim 17 or 18 or the composition of claim 22 in medicine.

26. Use of the IL-11Rα-binding protein of any one of claims 1 to 16 or 18 or the antibody of claim 17 or 18 in the manufacture of a medicament to treat an IL-11-mediated condition.

27. The IL-11Rα-binding protein of any one of claims 1 to 16 or 18 or the antibody of claim 17 or 18 or the composition of claim 22 for use in the treatment of an IL-11-mediated condition.

28. A method for localizing and/or detecting and/or diagnosing and/or prognosing an IL-11-mediated condition associated with a cell expressing IL-11Rα, the method comprising detecting *in vivo* the IL-11Rα-binding protein of claim 18 or the antibody of claim 18 bound to the IL-11Rα expressing cell, if present, wherein the IL-11Rα-binding protein or antibody is conjugated to a detectable tag.

29. The method of claim 28 additionally comprising administering the IL-11Rα-binding protein or antibody to the subject.

30. A method for detecting IL-11Rα or a cell expressing same in a sample, the method comprising contacting the sample with the IL-11Rα-binding protein of any one of claims 1 to 16 or 18 or the antibody of claim 17 or 18 such that a complex forms and detecting the complex, wherein detection of the complex is indicative of IL-11Rα or a cell expressing same in the sample.

31. A method for diagnosing or prognosing a IL-11Rα-mediated condition, the method comprising performing the method of claim 30 to detect IL-11Rα or a cell expressing same, wherein detection of the IL-11Rα or cell expressing same is diagnostic or prognostic of the condition.

32. The method of any one of claims 23, 28 or 31 or the use of claim 26 or the IL-11Rα-binding protein or the antibody or the composition for use of claim 27, wherein the IL-11-mediated condition is an autoimmune condition, an inflammatory condition, a wasting condition, a bone condition or a cancer.

8E2 L1	INNYLN
TS-303	VDYWVE
TS-305	VGIYVE
TS-306	VDKYVE
TS-307	VSMYVE
TS-310	VAMYIE
TS-311	VSQYIE
TS-312	IGQYVE
TS-313	VSGYVE
TS-322	VHHYME
Consensus	**VSXYVE**

8E2 L3.1	QQYDNL
TS-2	QQAEDQ
TS-4	QQHEFQ
TS-6	EQFESQ
TS-7	QQHENQ
TS-9	QQAEEQ
TS-13	QQNETQ
TS-14	QQHDNQ
TS-17	SQFESQ
TS-20	QQNESQ
TS-21	QQSESQ
TS-22	QQFETQ
TS-29	QQSEEQ
TS-32	TQNETQ
Consensus	**QQXESQ**

8E2 L3.2	DNLSPT
TS-49	ESQAPE
TS-51	ESQWPF
TS-55	ETQTPA
TS-57	ETQMPL
TS-58	ETQQPF
TS-63	DTQQPN
TS-64	ESQWPQ
Consensus	**ETQXPF**

8E2 H1	SWYSMT
TS-66	AWWSIA
TS-69	GWWSVT
TS-71	WRWSIT
TS-76	WRWSIT
TS-79	AWFSVT
TS-82	WRWSVT
TS-88	WRWSIT
TS-89	WRWSIT
TS-90	EWYSIT
TS-91	GWWSLT
TS-92	SWWSIT
Consensus	WWWSIT

8E2 H2	VPSGGH
TS-97	VPWADY
TS-101	VPWGDL
TS-103	VPYGDL
TS-104	VPWGHI
TS-107	VPWGDF
TS-108	VPWGHL
TS-115	VPHGDL
Consensus	VPWGDL

8E2 H3.1	GPGNGS
TS-129	PLDWGM
TS-133	PVDWGR
TS-134	PRDWGL
TS-135	PLDWGL
TS-138	PLDWGR
TS-140	PNDWGL
TS-143	PHDWGL
TS-151	PHDWGR
TS-156	PRDWGR
Consensus	PRDWGX

8E2 H3.2	WGSFDL
TS-213	WGQFAV
TS-214	WGSFWF
TS-215	WGSFWQ
TS-218	WGSFWE
TS-221	WGSFWY
TS-222	WGTFAY
TS-224	WGSFWT
Consensus	WGSFWY

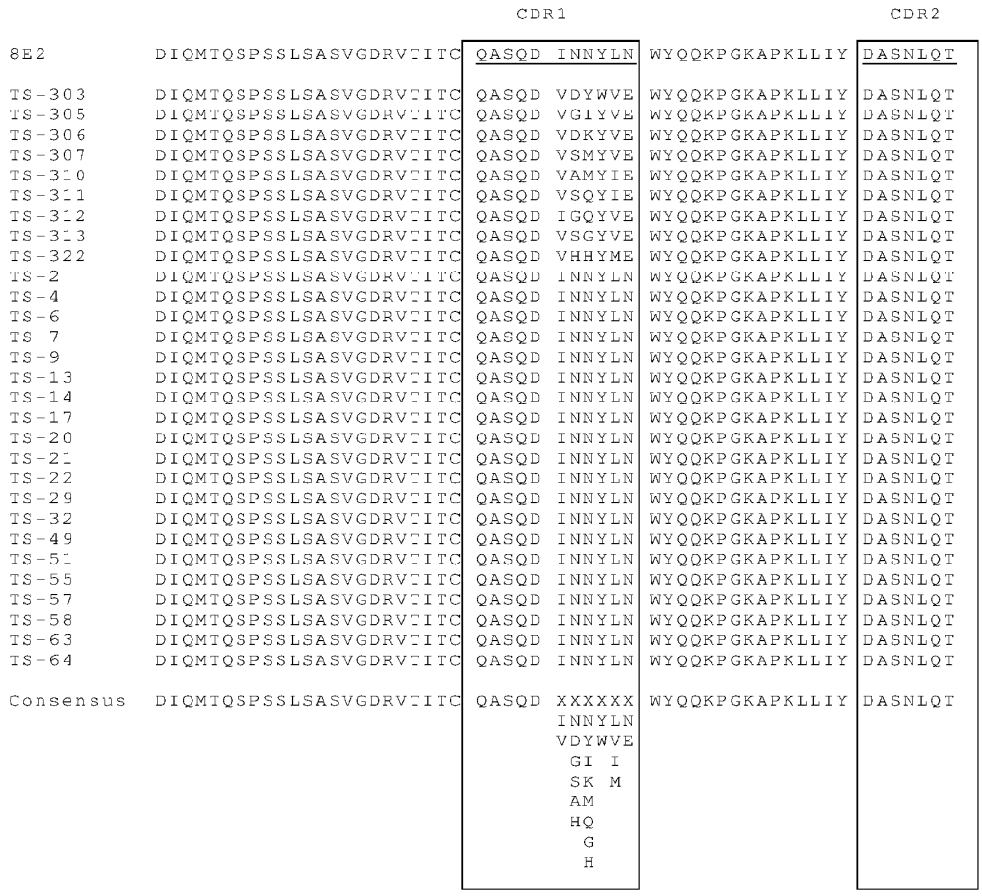

CDR1
CDR2
8E2 DIQMTQSPSSLSASVGDRVTITC QASQD INNYLN WYQQKPGKAPKLLIY DASNLQT
TS-303 DIQMTQSPSSLSASVGDRVTITC QASQD VDYWVE WYQQKPGKAPKLLIY DASNLQT
TS-305 DIQMTQSPSSLSASVGDRVTITC QASQD VGIYVE WYQQKPGKAPKLLIY DASNLQT
TS-306 DIQMTQSPSSLSASVGDRVTITC QASQD VDKYVE WYQQKPGKAPKLLIY DASNLQT
TS-307 DIQMTQSPSSLSASVGDRVTITC QASQD VSMYVE WYQQKPGKAPKLLIY DASNLQT
TS-310 DIQMTQSPSSLSASVGDRVTITC QASQD VAMYIE WYQQKPGKAPKLLIY DASNLQT
TS-311 DIQMTQSPSSLSASVGDRVTITC QASQD VSQYIE WYQQKPGKAPKLLIY DASNLQT
TS-312 DIQMTQSPSSLSASVGDRVTITC QASQD IGQYVE WYQQKPGKAPKLLIY DASNLQT
TS-313 DIQMTQSPSSLSASVGDRVTITC QASQD VSGYVE WYQQKPGKAPKLLIY DASNLQT
TS-322 DIQMTQSPSSLSASVGDRVTITC QASQD VHHYME WYQQKPGKAPKLLIY DASNLQT
TS-2 DIQMTQSPSSLSASVGDRVTITC QASQD INNYLN WYQQKPGKAPKLLIY DASNLQT
TS-4 DIQMTQSPSSLSASVGDRVTITC QASQD INNYLN WYQQKPGKAPKLLIY DASNLQT
TS-6 DIQMTQSPSSLSASVGDRVTITC QASQD INNYLN WYQQKPGKAPKLLIY DASNLQT
TS 7 DIQMTQSPSSLSASVGDRVTITC QASQD INNYLN WYQQKPGKAPKLLIY DASNLQT
TS-9 DIQMTQSPSSLSASVGDRVTITC QASQD INNYLN WYQQKPGKAPKLLIY DASNLQT
TS-13 DIQMTQSPSSLSASVGDRVTITC QASQD INNYLN WYQQKPGKAPKLLIY DASNLQT
TS-14 DIQMTQSPSSLSASVGDRVTITC QASQD INNYLN WYQQKPGKAPKLLIY DASNLQT
TS-17 DIQMTQSPSSLSASVGDRVTITC QASQD INNYLN WYQQKPGKAPKLLIY DASNLQT
TS-20 DIQMTQSPSSLSASVGDRVTITC QASQD INNYLN WYQQKPGKAPKLLIY DASNLQT
TS-21 DIQMTQSPSSLSASVGDRVTITC QASQD INNYLN WYQQKPGKAPKLLIY DASNLQT
TS-22 DIQMTQSPSSLSASVGDRVTITC QASQD INNYLN WYQQKPGKAPKLLIY DASNLQT
TS-29 DIQMTQSPSSLSASVGDRVTITC QASQD INNYLN WYQQKPGKAPKLLIY DASNLQT
TS-32 DIQMTQSPSSLSASVGDRVTITC QASQD INNYLN WYQQKPGKAPKLLIY DASNLQT
TS-49 DIQMTQSPSSLSASVGDRVTITC QASQD INNYLN WYQQKPGKAPKLLIY DASNLQT
TS-51 DIQMTQSPSSLSASVGDRVTITC QASQD INNYLN WYQQKPGKAPKLLIY DASNLQT
TS-55 DIQMTQSPSSLSASVGDRVTITC QASQD INNYLN WYQQKPGKAPKLLIY DASNLQT
TS-57 DIQMTQSPSSLSASVGDRVTITC QASQD INNYLN WYQQKPGKAPKLLIY DASNLQT
TS-58 DIQMTQSPSSLSASVGDRVTITC QASQD INNYLN WYQQKPGKAPKLLIY DASNLQT
TS-63 DIQMTQSPSSLSASVGDRVTITC QASQD INNYLN WYQQKPGKAPKLLIY DASNLQT
TS-64 DIQMTQSPSSLSASVGDRVTITC QASQD INNYLN WYQQKPGKAPKLLIY DASNLQT
Consensus DIQMTQSPSSLSASVGDRVTITC QASQD XXXXXX WYQQKPGKAPKLLIY DASNLQT
INNYLN
VDYWVE
GI I
SK M
AM
HQ
G
H

Figure 3A

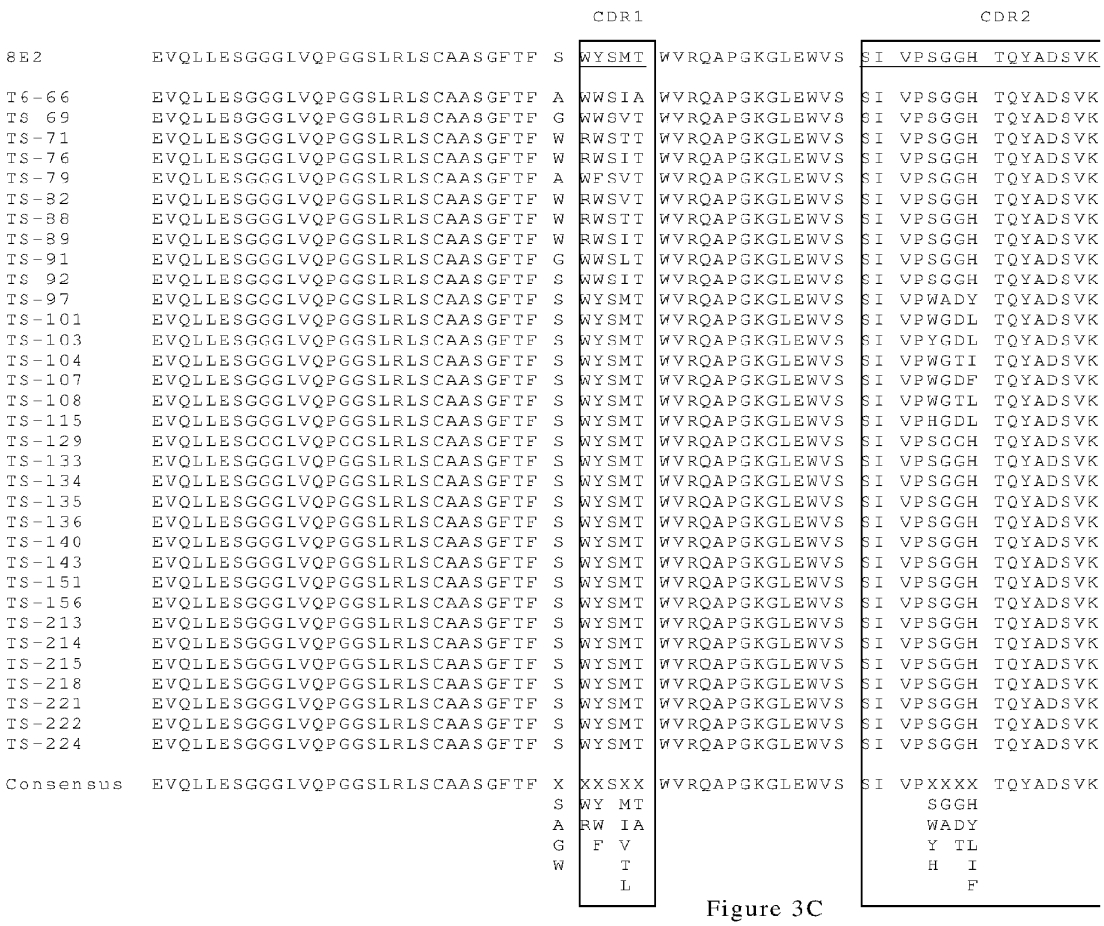
		CDR3		
8E2	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQYDNL SPT	FGPGTKVDIK	SEQ ID NO: 5
TS-3C3	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQYDNL SPT	FGPGTKVDIK	SEQ ID NO: 6
TS-3C5	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQYDNL SPT	FGPGTKVDIK	SEQ ID NO: 7
TS-3C6	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQYDNL SPT	FGPGTKVDIK	SEQ ID NO: 8
TS-3C7	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQYDNL SPT	FGPGTKVDIK	SEQ ID NO: 9
TS-310	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQYDNL SPT	FGPGTKVDIK	SEQ ID NO: 1
TS-311	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQYDNL SPT	FGPGTKVDIK	SEQ ID NO: 1
TS-312	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQYDNL SPT	FGPGTKVDIK	SEQ ID NO: 1
TS-313	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQYDNL SPT	FGPGTKVDIK	SEQ ID NO: 1
TS-322	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQYDNL SPT	FGPGTKVDIK	SEQ ID NO: 1
TS-2	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQAEDQ SPT	FGPGTKVDIK	SEQ ID NO: 1
TS-4	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQHEFQ SPT	FGPGTKVDIK	SEQ ID NO: 1
TS-6	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	EQFESQ SPT	FGPGTKVDIK	SEQ ID NO: 1
TS-7	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQHENQ SPT	FGPGTKVDIK	SEQ ID NO: 1
TS-9	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQAEEQ SPT	FGPGTKVDIK	SEQ ID NO: 1
TS-13	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQNETQ SPT	FGPGTKVDIK	SEQ ID NO: 2
TS-14	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQHDNQ SPT	FGPGTKVDIK	SEQ ID NO: 2
TS-17	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	SQFESQ SPT	FGPGTKVDIK	SEQ ID NO: 2
TS-20	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQNESQ SPT	FGPGTKVDIK	SEQ ID NO: 2
TS-21	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQSESQ SPT	FGPGTKVDIK	SEQ ID NO: 2
TS-22	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQFETQ SPT	FGPGTKVDIK	SEQ ID NO: 2
TS-29	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQSEEQ SPT	FGPGTKVDIK	SEQ ID NO: 2
TS-32	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	TQWETQ SPT	FGPGTKVDIK	SEQ ID NO: 2
TS-49	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQYESQ APE	FGPGTKVDIK	SEQ ID NO: 2
TS-51	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQYESQ WPF	FGPGTKVDIK	SEQ ID NO: 2
TS-55	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQYETQ TPA	FGPGTKVDIK	SEQ ID NO: 3
TS-57	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQYETQ MPL	FGPGTKVDIK	SEQ ID NO: 3
TS-58	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQYETQ QPF	FGPGTKVDIK	SEQ ID NO: 3
TS-63	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQYDTQ QPN	FGPGTKVDIK	SEQ ID NO: 3
TS-64	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQYESQ WPQ	FGPGTKVDIK	SEQ ID NO: 3
Consensus	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	XQXXXX XPX Q YDNL S T E AEDQ A E S H F W F T F S T A N E M L S T Q N W Q	FGPGTKVDIK	SEQ ID NO: 3

Figure 3A Continued

		CDR1		CDR2
8E2	DIQMTQSPSSLSASVGDRVTITC	QASQD INNYLN	WYQQKPGKAPKLLIY	DASNLQT
TS-306	DIQMTQSPSSLSASVGDRVTITC	QASQD VDKYVE	WYQQKPGKAPKLLIY	DASNLQT
TS-2	DIQMTQSPSSLSASVGDRVTITC	QASQD INNYLN	WYQQKPGKAPKLLIY	DASNLQT
TS-4	DIQMTQSPSSLSASVGDRVTITC	QASQD INNYLN	WYQQKPGKAPKLLIY	DASNLQT
TS-7	DIQMTQSPSSLSASVGDRVTITC	QASQD INNYLN	WYQQKPGKAPKLLIY	DASNLQT
TS-14	DIQMTQSPSSLSASVGDRVTITC	QASQD INNYLN	WYQQKPGKAPKLLIY	DASNLQT
TS-51	DIQMTQSPSSLSASVGDRVTITC	QASQD INNYLN	WYQQKPGKAPKLLIY	DASNLQT
Consensus	DIQMTQSPSSLSASVGDRVTITC	QASQD XXXYXX IDK LN VNN VE	WYQQKPGKAPKLLIY	DASNLQT

		CDR3		
8E2	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQYDNL SPT	FGPGTKVDIK	SEQ ID NO: 5
TS-306	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQYDNL SPT	FGPGTKVDIK	SEQ ID NO: 8
TS-2	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQAEDQ SPT	FGPGTKVDIK	SEQ ID NO: 1
TS-4	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQHEFQ SPT	FGPGTKVDIK	SEQ ID NO: 1
TS-7	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQHENQ SPT	FGPGTKVDIK	SEQ ID NO: 1
TS-14	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQHDNQ SPT	FGPGTKVDIK	SEQ ID NO: 2
TS-51	GVPSRFSGSGSCTDFTFTISSLQPEDIATYYC	QQYESQ WPF	FGPGTKVDIK	SEQ ID NO: 2
Consensus	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQXXXX XPX YDNL S T AEDQ W F H F N S	FGPGTKVDIK	SEQ ID NO: 3

Figure 3B


CDR1
CDR2
8E2 EVQLLESGGGLVQPGGSLRLSCAASGFTF S WYSMT WVRQAPGKGLEWVS SI VPSGGH TQYADSVK
T6-66 EVQLLESGGGLVQPGGSLRLSCAASGFTF A WWSIA WVRQAPGKGLEWVS SI VPSGGH TQYADSVK
TS 69 EVQLLESGGGLVQPGGSLRLSCAASGFTF G WWSVT WVRQAPGKGLEWVS SI VPSGGH TQYADSVK
TS-71 EVQLLESGGGLVQPGGSLRLSCAASGFTF W RWSTT WVRQAPGKGLEWVS SI VPSGGH TQYADSVK
TS-76 EVQLLESGGGLVQPGGSLRLSCAASGFTF W RWSIT WVRQAPGKGLEWVS SI VPSGGH TQYADSVK
TS-79 EVQLLESGGGLVQPGGSLRLSCAASGFTF A WFSVT WVRQAPGKGLEWVS SI VPSGGH TQYADSVK
TS-82 EVQLLESGGGLVQPGGSLRLSCAASGFTF W RWSVT WVRQAPGKGLEWVS SI VPSGGH TQYADSVK
TS-88 EVQLLESGGGLVQPGGSLRLSCAASGFTF W RWSTT WVRQAPGKGLEWVS SI VPSGGH TQYADSVK
TS-89 EVQLLESGGGLVQPGGSLRLSCAASGFTF W RWSIT WVRQAPGKGLEWVS SI VPSGGH TQYADSVK
TS-91 EVQLLESGGGLVQPGGSLRLSCAASGFTF G WWSLT WVRQAPGKGLEWVS SI VPSGGH TQYADSVK
TS 92 EVQLLESGGGLVQPGGSLRLSCAASGFTF S WWSIT WVRQAPGKGLEWVS SI VPSGGH TQYADSVK
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TS-101 EVQLLESGGGLVQPGGSLRLSCAASGFTF S WYSMT WVRQAPGKGLEWVS SI VPWGDL TQYADSVK
TS-103 EVQLLESGGGLVQPGGSLRLSCAASGFTF S WYSMT WVRQAPGKGLEWVS SI VPYGDL TQYADSVK
TS-104 EVQLLESGGGLVQPGGSLRLSCAASGFTF S WYSMT WVRQAPGKGLEWVS SI VPWGTI TQYADSVK
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TS-108 EVQLLESGGGLVQPGGSLRLSCAASGFTF S WYSMT WVRQAPGKGLEWVS SI VPWGTL TQYADSVK
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TS-224 EVQLLESGGGLVQPGGSLRLSCAASGFTF S WYSMT WVRQAPGKGLEWVS SI VPSGGH TQYADSVK
Consensus EVQLLESGGGLVQPGGSLRLSCAASGFTF X XXSXX WVRQAPGKGLEWVS SI VPXXXX TQYADSVK
S WY MT SGGH
A RW IA WADY
G F V Y TL
W T H I
L F

Figure 3C

		CDR3			
8E2	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	GPG	WGSFDL	WGRGTLVTVSS	SEQ ID NO:
T6-66	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	GPG	WGSFDL	WGRGTLVTVSS	SEQ ID NO:
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TS-71	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	GPG	WGSFDL	WGRGTLVTVSS	SEQ ID NO:
TS-76	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	GPG	WGSFDL	WGRGTLVTVSS	SEQ ID NO:
TS-79	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	GPG	WGSFDL	WGRGTLVTVSS	SEQ ID NO:
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TS-88	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	GPG	WGSFDL	WGRGTLVTVSS	SEQ ID NO:
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TS-91	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	GPG	WGSFDL	WGRGTLVTVSS	SEQ ID NO:
TS-92	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	GPG	WGSFDL	WGRGTLVTVSS	SEQ ID NO:
TS-97	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	GPG	WGSFDL	WGRGTLVTVSS	SEQ ID NO:
TS-101	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	GPG	WGSFDL	WGRGTLVTVSS	SEQ ID NO:
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TS-115	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	GPG	WGSFDL	WGRGTLVTVSS	SEQ ID NO:
TS-129	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	PED	WGMFDL	WGRGTLVTVSS	SEQ ID NO:
TS-133	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	PVD	WGRFDL	WGRGTLVTVSS	SEQ ID NO:
TS-134	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	PED	WGLFDL	WGRGTLVTVSS	SEQ ID NO:
TS-135	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	PLD	WGLFDL	WGRGTLVTVSS	SEQ ID NO:
TS-136	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	PLD	WGRFDL	WGRGTLVTVSS	SEQ ID NO:
TS-140	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	PND	WGLFDL	WGRGTLVTVSS	SEQ ID NO:
TS-143	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	PHD	WGLFDL	WGRGTLVTVSS	SEQ ID NO:
TS-151	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	PHD	WGRFDL	WGRGTLVTVSS	SEQ ID NO:
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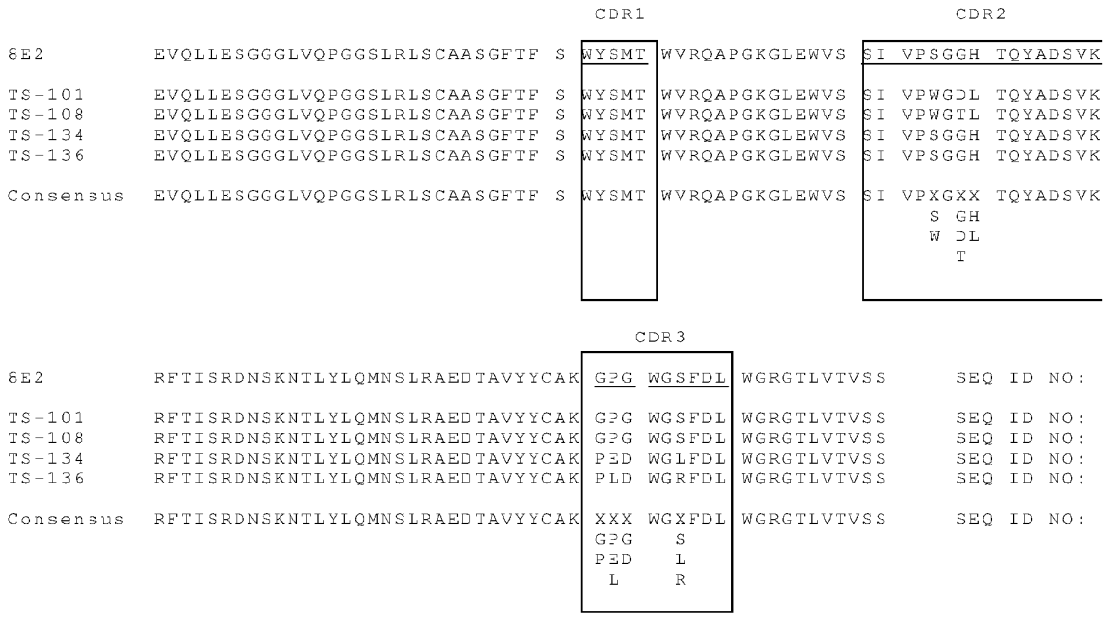
TS-213	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	GPG	WGQFAV	WGRGTLVTVSS	SEQ ID NO:
TS-214	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	GPG	WGSFWF	WGRGTLVTVSS	SEQ ID NO:
TS-215	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	GPG	WGSFWQ	WGRGTLVTVSS	SEQ ID NO:
TS-218	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	GPG	WGSFWE	WGRGTLVTVSS	SEQ ID NO:
TS-221	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	GPG	WGSFWY	WGRGTLVTVSS	SEQ ID NO:
TS 222	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	GPG	WGTFAY	WGRGTLVTVSS	SEQ ID NO:
TS-224	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	GPG	WGSFWT	WGRGTLVTVSS	SEQ ID NO:
Consensus	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	XXX	WGXFXX	WGRGTLVTVSS	SEQ ID NO:
		GPG	S DL		
		PED	M AV		
		V	R WF		
		L	L Q		
		N	Q E		
		H	T Y		
		E	T		

Figure 3C Conti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CDR2
8E2 EVQLLESGGGLVQPGGSLRLSCAASGFTF S WYSMT WVRQAPGKGLEWVS SI VPSGGH TQYADSVK
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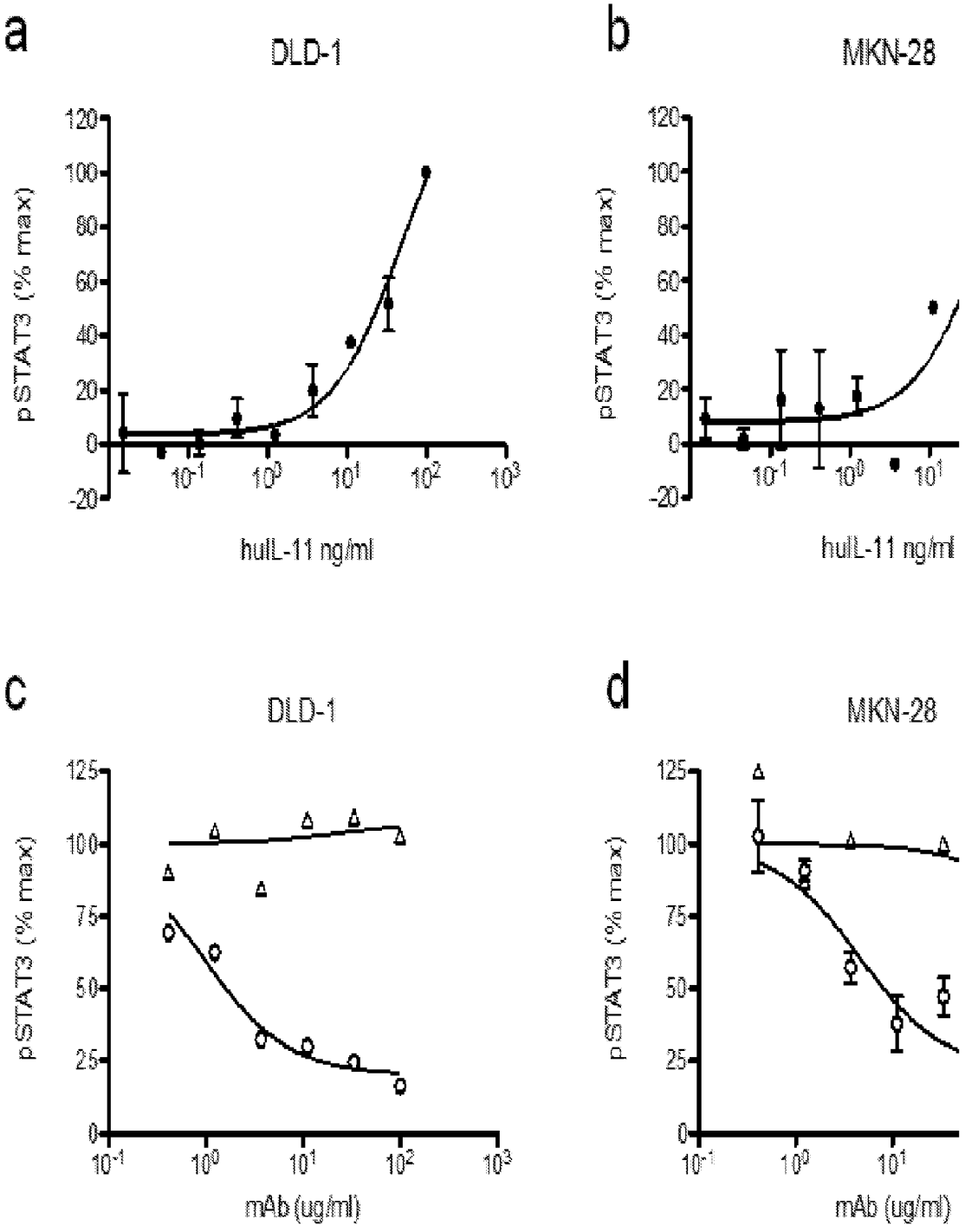
TS-108 EVQLLESGGGLVQPGGSLRLSCAASGFTF S WYSMT WVRQAPGKGLEWVS SI VPWGTL TQYADSVK
TS-134 EVQLLESGGGLVQPGGSLRLSCAASGFTF S WYSMT WVRQAPGKGLEWVS SI VPSGGH TQYADSVK
TS-136 EVQLLESGGGLVQPGGSLRLSCAASGFTF S WYSMT WVRQAPGKGLEWVS SI VPSGGH TQYADSVK
Consensus EVQLLESGGGLVQPGGSLRLSCAASGFTF S WYSMT WVRQAPGKGLEWVS SI VPXGXX TQYADSVK
S GH
W DL
T
CDR3
8E2 RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK GPG WGSFDL WGRGTLVTVSS SEQ ID NO:
TS-101 RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK GPG WGSFDL WGRGTLVTVSS SEQ ID NO:
TS-108 RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK GPG WGSFDL WGRGTLVTVSS SEQ ID NO:
TS-134 RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK PED WGLFDL WGRGTLVTVSS SEQ ID NO:
TS-136 RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK PLD WGRFDL WGRGTLVTVSS SEQ ID NO:
Consensus RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK XXX WGXFDL WGRGTLVTVSS SEQ ID NO:
GPG S
PED L
L R

Figure 3D


a
DLD-1
pSTAT3 (% max)
huIL-11 ng/ml
b
MKN-28
pSTAT3 (% max)
huIL-11 ng/ml
c
DLD-1
pSTAT3 (% max)
mAb (ug/ml)
d
MKN-28
pSTAT3 (% max)
mAb (ug/ml)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2014/000083

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/24 (2006.01) A61K 39/395 (2006.01) A61P 15/18 (2006.01) A61P 29/00 (2006.01)

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)

EPODOC, WPI, Medline, GenomeQuest

Keywords: IL-11Ralpha, CRSDA, Interleukin 11 receptor alpha, antibod+, immunoglob+, +scfv, diabod+

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

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"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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 June 2014	13 June 2014
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Johanna Lowery AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0262832968

Form PCT/ISA/210 (fifth sheet) (July 2009)

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/AU2014/000083**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	US 2009/0202533 A1 (BACA et al) 13 August 2009 [0140]-[0141] Whole Document	2 1, 3-32
A	BLANC, C. et al "monoclonal antibodies against the human interleukin-11 receptor alpha-chain (IL-11Rα) and their use in studies of human mononuclear cells." Journal of Immunological Mehtods, Vol. 241, page 43-59. 31/7/2000. Whole Document	1-32

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2014/000083**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
US 2009/0202533 A1	13 August 2009	US 8182814 B2	22 May 2012

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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22 June 2017 (22.06.2017)

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C07K 16/28 (2006.01)

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(22) International Filing Date:
16 December 2016 (16.12.2016)

(25) Filing Language: English

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(72) Inventors: **COOK, Stuart Alexander**; 6 Sunset Square, Clementi Park, Singapore 597304 (SG). **SCHAEFER, Sebastian**; 76 Shenton Way, 76 Shenton #24-04, Singapore 079119 (SG).

(74) Agents: **CLEGG, Richard** et al.; Mewburn Ellis LLP, City Tower, 40 Basinghall Street, London, Greater London EC2V 5DE (GB).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,

[Continued on next page]

(54) Title: TREATMENT OF FIBROSIS

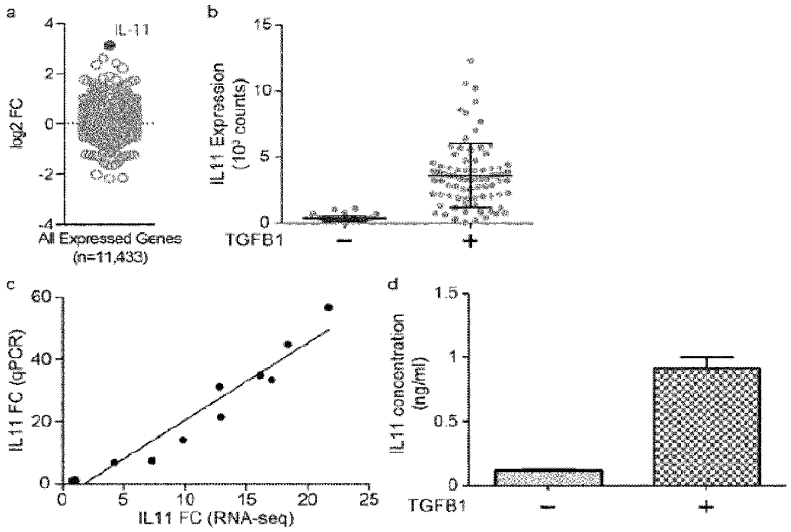

Figure 1

(57) Abstract: The treatment, prevention or alleviation of fibrosis in a subject through the administration of an agent capable of inhibiting the action of Interleukin 11 (IL-11) is disclosed.

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SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

Treatment of Fibrosis

Field of the Invention

The present invention relates to the diagnosis and treatment of fibrosis.

Background to the Invention

Fibrosis is an essential process that is a critical part of wound healing. Excessive fibrosis is common in many rare and common disease conditions and is important in disease pathogenesis. Diseases characterized by excessive fibrosis include but are not restricted to: systemic sclerosis, scleroderma, hypertrophic cardiomyopathy, dilated cardiomyopathy (DCM), atrial fibrillation, ventricular fibrillation, myocarditis, liver cirrhosis, kidney diseases, diseases of the eye, asthma, cystic fibrosis, arthritis and idiopathic pulmonary fibrosis. Despite the large impact on human health, therapeutic and diagnostic approaches to fibrosis are still an unmet medical need.

The real physiological role of Interleukin 11 (IL-11) remains unclear. IL-11 has been most strongly linked with activation of haematopoetic cells and with platelet production but also found to be pro- as well as anti-inflammatory, pro-angiogenic and important for neoplasia. It is known that TGFβ1 or tissue injury can induce IL-11 expression (Zhu, M. et al. IL-11 Attenuates Liver Ischemia/Reperfusion Injury (IRI) through STAT3 Signaling Pathway in Mice. PLOS ONE 10, (2015); Yashiro, R. et al. Transforming growth factor-beta stimulates interleukin-11 production by human periodontal ligament and gingival fibroblasts. J. Clin. Periodontol. 33, 165–71 (2006); Obana, M. et al. Therapeutic activation of signal transducer and activator of transcription 3 by interleukin-11 ameliorates cardiac fibrosis after myocardial infarction. Circulation 121, 684–91 (2010); Tang, W., Yang, L., Yang, Y. C., Leng, S. X. & Elias, J. A. Transforming growth factor-beta stimulates interleukin-11 transcription via complex activating protein-1-dependent pathways. J. Biol. Chem. 273, 5506–13 (1998)).

The role for IL-11 in fibrosis is not clear from the published literature. IL-11 is thought to be important for fibrosis and inflammation in the lung (Tang, W. et al. Targeted expression of IL-11 in the murine airway causes lymphocytic inflammation, bronchial remodeling, and airways obstruction. J. Clin. Invest. 98, 2845–53 (1996)) and its expression level is correlated with collagen levels in the skin (Toda, M. et al. Polarized in vivo expression of IL-11 and IL-17 between acute and chronic skin lesions. Journal of

Allergy and Clinical Immunology 111, 875–881 (2003)) and the respiratory system (Molet, S., Hamid, Q. & Hamilos, D. IL-11 and IL-17 expression in nasal polyps: Relationship to collagen deposition and suppression by intranasal fluticasone propionate. The Laryngoscope 113, (2003); Minshall et al. IL-11 expression is increased in severe asthma: association with epithelial cells and eosinophils. The Journal of allergy and clinical immunology 105, (2000)).

However, the majority of studies suggest that IL-11 is anti-fibrotic: in the heart (Obana, M. et al. Therapeutic activation of signal transducer and activator of transcription 3 by interleukin-11 ameliorates cardiac fibrosis after myocardial infarction. Circulation 121, 684–91 (2010); Obana, M. et al. Therapeutic administration of IL-11 exhibits the postconditioning effects against ischemia-reperfusion injury via STAT3 in the heart. American Journal of Physiology. Heart and circulatory physiology 303, H569–77 (2012)) and kidney (Stangou, M. et al. Effect of IL-11 on glomerular expression of TGF-beta and extracellular matrix in nephrotoxic nephritis in Wistar Kyoto rats. Journal of nephrology 24, 106–11 (2011); Ham, A. et al. Critical role of interleukin-11 in isoflurane-mediated protection against ischemic acute kidney injury in mice. Anesthesiology 119, 1389–401 (2013)) and anti-inflammatory in several tissues and chronic inflammatory diseases (Trepicchio & Dorner. The therapeutic utility of Interleukin-11 in the treatment of inflammatory disease. (1998). doi:10.1517/13543784.7.9.1501). The molecular mode of action of IL-11 in general, is thought to be regulation of RNA expression of mRNA levels via STAT3-mediated transcription (Zhu, M. et al. IL-11 Attenuates Liver Ischemia/Reperfusion Injury (IRI) through STAT3 Signaling Pathway in Mice. PLOS ONE 10, (2015)).

Summary of the Invention

One aspect of the present invention concerns the treatment, prevention or alleviation of fibrosis in a subject in need of treatment through the administration of an agent capable of inhibiting the action of Interleukin 11 (IL-11). The inventors have identified IL-11 to have a pro-fibrotic action. The present invention is particularly concerned with inhibiting the pro-fibrotic action of IL-11. Embodiments of the invention concern inhibition or prevention of the IL-11 mediated pro-fibrotic signal, e.g. as mediated by binding of IL-11 to an IL-11 receptor.

In some embodiments an agent capable of inhibiting the action of IL-11 may prevent or reduce the binding of IL-11 to an IL-11 receptor.

In some embodiments an agent capable of inhibiting the action of IL-11 may bind IL-11 to form a complex comprising the agent and IL-11. The complex may be a non-covalent or covalent complex. In some embodiments, the formation of the agent:IL-11 complex may prevent or reduce the ability of IL-11 to bind to an IL-11 receptor. In some embodiments such prevention or reduction may be the result of a reduction of the productive binding of IL-11 to an IL-11 receptor, i.e. reduction in the ability of IL-11 to initiate IL-11 receptor mediated signalling. In some embodiments formation of the agent:IL-11 complex may sequester IL-11 away from the IL-11 receptor, thereby preventing or reducing the contact of IL-11 with an IL-11 receptor and/or preventing or reducing the amount of IL-11 available for binding to an IL-11 receptor. In some embodiments the agent may be a decoy receptor.

In some embodiments an agent capable of inhibiting the action of IL-11 may bind to an IL-11 receptor. An agent that binds an IL-11 receptor may prevent or reduce the ability of IL-11 to bind to an IL-11 receptor (IL-11R).

Another aspect of the present invention concerns the treatment, prevention or alleviation of fibrosis in a subject in need of treatment through the administration of an agent capable of preventing or reducing the expression of IL-11 or an IL-11 receptor (IL-11R).

In one aspect of the present invention an agent capable of inhibiting the action of Interleukin 11 (IL-11) for use in a method of treating or preventing fibrosis is provided.

In another aspect of the present invention the use of an agent capable of inhibiting the action of IL-11 in the manufacture of a medicament for use in a method of treating or preventing fibrosis is provided.

In another aspect of the present invention a method of treating or preventing fibrosis is provided, the method comprising administering to a subject in need of treatment a therapeutically effective amount of an agent capable of inhibiting the action of IL-11.

In some embodiments the agent capable of inhibiting the action of IL-11 is an agent capable of preventing or reducing the binding of IL-11 to an IL-11 receptor.

In some embodiments the agent capable of inhibiting the action of IL-11 is an IL-11 binding agent. IL-11 binding agents may be selected from the group consisting of: an antibody, polypeptide, peptide, oligonucleotide, aptamer or small molecule. In some embodiments the IL-11 binding agent is an antibody. In some embodiments the IL-11 binding agent is a decoy receptor.

In some embodiments the agent capable of inhibiting the action of IL-11 is an IL-11 receptor (IL-11R) binding agent. IL-11R binding agents may be selected from the group consisting of: an antibody, polypeptide, peptide, oligonucleotide, aptamer or small molecule. In some embodiments the IL-11R binding agent is an antibody.

In another aspect of the present invention an agent capable of preventing or reducing the expression of IL-11 or IL-11R for use in a method of treating or preventing fibrosis is provided.

In another aspect of the present invention the use of an agent capable of preventing or reducing the expression of IL-11 or IL-11R in the manufacture of a medicament for use in a method of treating or preventing fibrosis is provided.

In another aspect of the present invention a method of treating or preventing fibrosis is provided, the method comprising administering to a subject in need of treatment a therapeutically effective amount of an agent capable of preventing or reducing the expression of IL-11 or IL-11R.

In some embodiments the agent capable of preventing or reducing the expression of IL-11 or IL-11R is a small molecule or oligonucleotide.

In some embodiments the fibrosis to be treated or prevented is fibrosis of the heart, liver or kidney. In some embodiments the fibrosis to be treated or prevented is fibrosis of the eye. In some embodiments the fibrosis is in the heart and is associated with dysfunction of the musculature or electrical properties of the heart, or thickening of the walls or valves of the heart. In some embodiments the fibrosis is in the liver and is associated with

chronic liver disease or liver cirrhosis. In some embodiments the fibrosis is in the kidney and is associated with chronic kidney disease.

In some embodiments the method of treating or preventing comprises administering a said agent to a subject in which IL-11 or IL-11R expression is upregulated. In some embodiments the method of treating or preventing comprises administering a said agent to a subject in which IL-11 or IL-11R expression has been determined to be upregulated. In some embodiments the method of treating or preventing comprises determining whether IL-11 or IL-11R expression is upregulated in the subject and administering a said agent to a subject in which IL-11 or IL-11R expression is upregulated.

In another aspect of the present invention a method of determining the suitability of a subject for the treatment or prevention of fibrosis with an agent capable of inhibiting the action of IL-11 is provided, the method comprising determining, optionally *in vitro*, whether IL-11 or IL-11R expression is upregulated in the subject.

In another aspect of the present invention a method of selecting a subject for the treatment or prevention of fibrosis with an agent capable of inhibiting the action of IL-11 is provided, the method comprising determining, optionally *in vitro*, whether IL-11 or IL-11R expression is upregulated in the subject.

In another aspect of the present invention a method of diagnosing fibrosis or a risk of developing fibrosis in a subject is provided, the method comprising determining, optionally *in vitro*, the upregulation of IL-11 or IL-11R in a sample obtained from the subject.

In some embodiments the method is a method of confirming a diagnosis of fibrosis in a subject suspected of having fibrosis.

In some embodiments the method further comprises selecting the subject for treatment with an agent capable of inhibiting the action of IL-11 or with an agent capable of preventing or reducing the expression of IL-11 or IL-11R.

In another aspect of the present invention a method of providing a prognosis for a subject having, or suspected of having fibrosis, is provided, the method comprising determining,

optionally *in vitro*, whether IL-11 or IL-11R is upregulated in a sample obtained from the subject and, based on the determination, providing a prognosis for treatment of the subject with an agent capable of inhibiting the action of IL-11 or with an agent capable of preventing or reducing the expression of IL-11 or IL-11R.

The method may further comprise selecting a subject determined to have upregulated IL-11 or IL-11R for treatment with an agent capable of inhibiting the action of IL-11 or with an agent capable of preventing or reducing the expression of IL-11 or IL-11R.

In another aspect of the present invention a method of diagnosing fibrosis or a risk of developing fibrosis in a subject is provided, the method comprising determining, optionally *in vitro*, one or more genetic factors in the subject that are predictive of upregulation of IL-11 or IL-11R expression, or of upregulation of IL-11 or IL-11R activity.

In some embodiments the method is a method of confirming a diagnosis of fibrosis in a subject suspected of having fibrosis.

In some embodiments the method further comprises selecting the subject for treatment with an agent capable of inhibiting the action of IL-11 or with an agent capable of preventing or reducing the expression of IL-11 or IL-11R.

In another aspect of the present invention a method of providing a prognosis for a subject having, or suspected of having, fibrosis, is provided, the method comprising determining, optionally *in vitro*, one or more genetic factors in the subject that are predictive of upregulation of IL-11 or IL-11R expression, or of upregulation of IL-11 or IL-11R activity.

In another aspect, of the present invention a method of treating fibrosis in a human subject is provided, the method comprising administering to a human subject in need of treatment a therapeutically effective amount of an anti-interleukin 11 (IL-11) antibody, wherein the anti-IL-11 antibody binds to IL-11 and inhibits IL-11 mediated signalling

In another aspect, of the present invention a method of treating fibrosis in a subject is provided, the method comprising:

(i) determining, optionally *in vitro*, whether IL-11 or an Interleukin 11 receptor (IL-11R) expression is upregulated in the subject; and

(ii) administering to a subject in which IL-11 or IL-11R expression is upregulated a therapeutically effective amount of an anti-IL-11 antibody, wherein the anti-IL-11 antibody binds to IL-11 and inhibits IL-11 mediated signalling.

In another aspect, of the present invention a method of treating fibrosis in a subject is provided, the method comprising:

(i) determining, optionally *in vitro*, one or more genetic factors in the subject that are predictive of upregulation of Interleukin 11 (IL-11) or Interleukin 11 receptor (IL-11R) expression or activity;

(ii) selecting a subject for treatment based on the determination in (i); and

(ii) administering to the selected subject a therapeutically effective amount of an anti-IL-11 antibody, wherein the anti-IL-11 antibody binds to IL-11 and inhibits IL-11 mediated signalling.

Description

IL-11 and IL-11 receptor

Interleukin 11 (IL-11), also known as adipogenesis inhibitory factor, is a pleiotropic cytokine and a member of the IL-6 family of cytokines that includes IL-6, IL-11, IL-27, IL-31, oncostatin, leukemia inhibitory factor (LIF), cardiotrophin-1 (CT-1), cardiotrophin-like cytokine (CLC), ciliary neurotrophic factor (CNTF) and neuropoetin (NP-1).

IL-11 is transcribed with a canonical signal peptide that ensures efficient secretion from cells. The immature form of human IL-11 is a 199 amino acid polypeptide whereas the mature form of IL-11 encodes a protein of 178 amino acid residues (Garbers and Scheller., Biol. Chem. 2013; 394(9):1145-1161). The human IL-11 amino acid sequence is available under UniProt accession no. P20809 (P20809.1 GI:124294). Recombinant human IL-11 (oprelvekin) is also commercially available. IL-11 from other species, including mouse, rat, pig, cow, several species of bony fish and primates, have also been cloned and sequenced.

In this specification IL-11 refers to an IL-11 from any species and includes isoforms, fragments, variants or homologues of an IL-11 from any species. In preferred embodiments the species is human (*Homo sapiens*). Isoforms, fragments, variants or homologues of an IL-11 may optionally be characterised as having at least 70%, preferably one of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or

100% amino acid sequence identity to the amino acid sequence of immature or mature IL-11 from a given species, e.g. human. Isoforms, fragments, variants or homologues of an IL-11 may optionally be characterised by ability to bind IL-11Rα (preferably from the same species) and stimulate signal transduction in cells expressing IL-11Rα and gp130 (e.g. as described in Curtis et al. Blood, 1997, 90(11); or Karpovich et al. Mol. Hum. Reprod. 2003 9(2): 75-80). A fragment of IL-11 may be of any length (by number of amino acids), although may optionally be at least 25% of the length of mature IL-11 and may have a maximum length of one of 50%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the length of mature IL-11. A fragment of IL-11 may have a minimum length of 10 amino acids, and a maximum length of one of 15, 20, 25, 30, 40, 50, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 or 195 amino acids

IL-11 signals through a homodimer of the ubiquitously expressed β-receptor glycoprotein 130 (gp130; also known as glycoprotein 130, IL6ST, IL6-beta or CD130). Gp130 is a transmembrane protein that forms one subunit of the type I cytokine receptor with the IL-6 receptor family. Specificity is gained through an individual IL-11 α-receptor (IL-11Rα), which does not directly participate in signal transduction, although the initial cytokine binding event to the α-receptor leads to the final complex formation with the β-receptors. IL-11 activates a downstream signaling pathway, which is predominantly the mitogen-activated protein kinase (MAPK)-cascade and the Janus kinase/signal transducer and activator of transcription (Jak/STAT) pathway (Garbers and Scheller, *supra*).

Human gp130 (including the 22 amino acid signal peptide) is a 918 amino acid protein, and the mature form is 866 amino acids, comprising a 597 amino acid extracellular domain, a 22 amino acid transmembrane domain, and a 277 amino acid intracellular domain. The extracellular domain of the protein comprises the cytokine-binding module (CBM) of gp130. The CBM of gp130 comprises the Ig-like domain D1, and the fibronectin-type III domains D2 and D3 of gp130. The amino acid sequence of human gp130 is available from Genbank accession no. NP_002175.2.

Human IL-11Rα is a 422 amino acid polypeptide (Genbank accession no. NP_001136256.1 GI:218505839) and shares ~85% nucleotide and amino acid sequence identity with the murine IL-11Rα (Du and Williams., Blood Vol, 89, No,11, June 1, 1997). Two isoforms of IL-11Rα have been reported, which differ in the cytoplasmic domain (Du and Williams, *supra*). The IL-11 receptor α-chain (IL-11Rα) shares many structural and functional similarities with the IL-6 receptor α-chain (IL-6Rα). The extracellular domain

shows 24% amino acid identity including the characteristic conserved Trp-Ser-X-Trp-Ser (WSXWS) motif. The short cytoplasmic domain (34 amino acids) lacks the Box 1 and 2 regions that are required for activation of the JAK/STAT signaling pathway.

IL-11Rα binds its ligand with a low affinity (Kd ~10 nmol/L) and alone is insufficient to transduce a biological signal. The generation of a high affinity receptor (Kd ~400 to 800 pmol/L) capable of signal transduction requires co-expression of the IL-11Rα and gp130 (Curtis et al (Blood 1997 Dec 1;90 (11):4403-12; Hilton et al., EMBO J 13:4765, 1994; Nandurkar et al., Oncogene 12:585, 1996). Binding of IL-11 to cell-surface IL-11Rα induces heterodimerization, tyrosine phosphorylation, activation of gp130 and MAPK and/or Jak/STAT signalling as described above.

The receptor binding sites on murine IL-11 have been mapped and three sites – sites I, II and III - identified. Binding to gp130 is reduced by substitutions in the site II region and by substitutions in the site III region. Site III mutants show no detectable agonist activity and have IL-11Rα antagonist activity (Cytokine Inhibitors Chapter 8; edited by Gennaro Ciliberto and Rocco Savino, Marcel Dekker, Inc. 2001).

In principle, a soluble IL-11Rα can also form biologically active soluble complexes with IL-11 (Pflanz et al., 1999 FEBS Lett, 450, 117-122) raising the possibility that, similar to IL-6, IL-11 may in some instances bind soluble IL-11Rα prior to binding cell-surface gp130 (Garbers and Scheller, *supra*). Curtis et al (Blood 1997 Dec 1;90 (11):4403-12) describe expression of a soluble murine IL-11 receptor alpha chain (sIL-11R) and examined signaling in cells expressing gp130. In the presence of gp130 but not transmembrane IL-11R the sIL-11R mediated IL-11 dependent differentiation of M1 leukemic cells and proliferation in Ba/F3 cells and early intracellular events including phosphorylation of gp130, STAT3 and SHP2 similar to signalling through transmembrane IL-11R.

In this specification an IL-11 receptor (IL-11R) refers to a polypeptide capable of binding IL-11 and inducing signal transduction in cells expressing gp130. An IL-11 receptor may be from any species and includes isoforms, fragments, variants or homologues of an IL-11 receptor from any species. In preferred embodiments the species is human (*Homo sapiens*). In some embodiments the IL-11 receptor may be IL-11Rα. Isoforms, fragments, variants or homologues of an IL-11Rα may optionally be characterised as having at least 70%, preferably one of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,

99% or 100% amino acid sequence identity to the amino acid sequence of IL-11Rα from a given species, e.g. human. Isoforms, fragments, variants or homologues of an IL-11Rα may optionally be characterised by ability to bind IL-11 (preferably from the same species) and stimulate signal transduction in cells expressing the IL-11Rα and gp130 (e.g. as described in Curtis et al. Blood, 1997, 90(11) or Karpovich et al. Mol. Hum. Reprod. 2003 9(2): 75-80). A fragment of an IL-11 receptor may be of any length (by number of amino acids), although may optionally be at least 25% of the length of the mature IL-11Rα and have a maximum length of one of 50%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the length of the mature IL-11Rα. A fragment of an IL-11 receptor fragment may have a minimum length of 10 amino acids, and a maximum length of one of 15, 20, 25, 30, 40, 50, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 400, or 415 amino acids.

Agent capable of inhibiting the action of IL-11

The IL-11 signaling pathway offers multiple routes for inhibition of IL-11 signaling. For example, inhibition may be achieved by preventing or reducing the binding of IL-11 to an IL-11 receptor. As a result, suitable agents may target either IL-11 or its receptor.

In some embodiments agents capable of inhibiting the action of IL-11 may bind to IL-11 and prevent or reduce IL-11 mediated signalling, e.g. through an IL-11 receptor. In some embodiments agents capable of inhibiting the action of IL-11 may bind to the IL-11 receptor and prevent or reduce IL-11 stimulated signalling.

Agents that bind to IL-11 may inhibit IL-11 mediated signalling by blocking the binding of IL-11 to an IL-11 receptor and/or by reducing the amount of IL-11 available to bind to its receptor. Suitable IL-11 binding agents may be IL-11 inhibitors or IL-11 antagonists.

IL-11 binding agents, e.g. anti-IL-11 antibodies, according to the present invention may exhibit at least one of the following properties:

a) Bind to human IL-11 with a K_D of 1μM or less, preferably one of ≤ 1μM, ≤ 100nM, ≤10nM, ≤1nM or ≤100pM;
b) Inhibit IL-11 mediated signalling via the IL-11Rα receptor, e.g. in a cell based assay in which the cells co-express IL-11Rα and gp130. Suitable cell based assays are ^{3}H-thymidine incorporation and Ba/F3 cell proliferation assays

described in e.g. Curtis et al. Blood, 1997, 90(11) and Karpovich et al. Mol. Hum. Reprod. 2003 9(2): 75-80. For example, IC_{50} for an IL-11 binding agent may be determined by culturing Ba/F3 cells expressing IL-11Rα and gp130 in the presence of human IL-11 and the IL-11 binding agent, and measuring ^{3}H-thymidine incorporation into DNA. Suitable IL-11 binding agents may exhibit an IC_{50} of 10 µg/ml or less, preferably one of ≤ 5 µg/ml, ≤ 4 µg/ml, ≤ 3.5 µg/ml, ≤ 3 µg/ml, ≤ 2 µg/ml, ≤ 1 µg/ml, ≤ 0.9 µg/ml, ≤ 0.8 µg/ml, ≤ 0.7 µg/ml, ≤ 0.6 µg/ml, or ≤ 0.5 µg/ml in such an assay.

c) Inhibit fibroblast proliferation, e.g. proliferation of cardiac/atrial fibroblasts. This can, for example, be evaluated in an assay wherein fibroblasts are stimulated with IL-11 or TGFβ1 and cell proliferation is monitored as described herein.

d) Inhibit myofibroblast generation, e.g. from cardiac/atrial fibroblasts. This can, for example, be evaluated in an assay wherein fibroblasts are stimulated with IL-11 or TGFβ1 and myofibroblast generation is monitored, e.g. by measuring αSMA levels.

e) Inhibit extracellular matrix production by fibroblasts, e.g. cardiac/atrial fibroblasts. This can, for example, be evaluated in an assay wherein fibroblasts are stimulated with IL-11 or TGFβ1 and production of extracellular matrix components is measured.

f) Inhibit collagen and/or periostin gene or protein expression in fibroblasts, e.g. cardiac/atrial fibroblasts. This can, for example, be evaluated in an assay wherein fibroblasts are stimulated with IL-11 or TGFβ1 and collagen and/or periostin gene or protein expression is measured.

IL-11 binding agents may be of any kind, but in some embodiments an IL-11 binding agent may be an antibody, polypeptide, peptide, oligonucleotide, aptamer or small molecule.

Suitable anti-IL-11 antibodies will preferably bind to IL-11 (the antigen), preferably human IL-11, and may have a dissociation constant (K_D) of one of ≤ 1µM, ≤ 100nM, ≤10nM, ≤1nM or ≤100pM. Binding affinity of an antibody for its target is often described in terms of its dissociation constant (K_D). Binding affinity can be measured by methods known in the art, such as by Surface Plasmon Resonance (SPR), or by a radiolabeled antigen binding assay (RIA) performed with the Fab version of the antibody and antigen molecule.

Anti-IL-11 antibodies may be antagonist antibodies that inhibit or reduce a biological activity of IL-11.

Anti-IL-11 antibodies may be neutralising antibodies that neutralise the biological effect of IL-11, e.g. its ability to stimulate productive signalling via an IL-11 receptor.

Neutralising activity may be measured by ability to neutralise IL-11 induced proliferation in the T11 mouse plasmacytoma cell line (Nordan, R. P. *et al*. (1987) J. Immunol. **139**:813).

Examples of known anti-IL-11 antibodies include monoclonal antibody clone 6D9A, clone KT8 (Abbiotec), clone M3103F11 (BioLegend), clone 1F1, clone 3C6 (Abnova Corporation), clone GF1 (LifeSpan Biosciences), clone 13455 (Source BioScience) and clone 22626 (R & D Systems, used in Bockhorn et al. Nat. Commun. (2013) 4(0):1393; Monoclonal Mouse IgG_{2A}; Catalog No. MAB218; R&D Systems, MN, USA).

Antibodies may optionally be selected to exhibit substantially no cross-reactivity with one or more of human, e.g. recombinant human, IL-6, CNTF, LIF, OSM, CLC or CT-1.

Peptide or polypeptide based IL-11 binding agents may be based on the IL-11 receptor, e.g. a IL-11 binding fragment of an IL-11 receptor. In one embodiment, suitable IL-11 binding agents may comprise an IL-11 binding fragment of the IL-11Rα chain, and may preferably be soluble and/or exclude one or more, or all, of the transmembrane domain(s). Such molecules may be described as decoy receptors.

Curtis et al (Blood 1997 Dec 1;90 (11):4403-12) report that a soluble murine IL-11 receptor alpha chain (sIL-11R) was capable of antagonizing the activity of IL-11 when tested on cells expressing the transmembrane IL-11R and gp130. They proposed that the observed IL-11 antagonism by the sIL-11R depends on limiting numbers of gp130 molecules on cells already expressing the transmembrane IL-11R.

The use of soluble decoy receptors as the basis for inhibition of signal transduction and therapeutic intervention has also been reported for other signalling molecule:receptor pairs, e.g. VEGF and the VEGF receptor (De-Chao Yu et al., Molecular Therapy (2012); 20 5, 938-947; Konner and Dupont Clin Colorectal Cancer 2004 Oct;4 Suppl 2:S81-5).

As such, in some embodiments an IL-11 binding agent may be provided in the form of a decoy receptor, e.g. a soluble IL-11 receptor. Competition for IL-11 provided by a decoy receptor has been reported to lead to IL-11 antagonist action (Curtis et al., *supra*).

Decoy IL-11 receptors preferably bind IL-11 and/or IL-11 containing complexes, and thereby make these species unavailable for binding to gp130, IL-11Rα and/or gp130:IL-11Rα receptors. As such, they act as 'decoy' receptors for IL-11 and IL-11 containing complexes, much in the same way that etanercept acts as a decoy receptor for TNFα. IL-11 mediated signalling is reduced as compared to the level of signalling in the absence of the decoy receptor.

Decoy IL-11 receptors preferably bind to IL-11 through one or more cytokine binding modules (CBMs). The CBMs are, or are derived from or homologous to, the CBMs of naturally occurring receptor molecules for IL-11. For example, decoy IL-11 receptors may comprise, or consist of, one or more CBMs which are from, are derived from or homologous to the CBM of gp130 and/or IL-11Rα.

In some embodiments, a decoy IL-11 receptor may comprise, or consist of, an amino acid sequence corresponding to the cytokine binding module of gp130. In some embodiments, a decoy IL-11 receptor may comprise an amino acid sequence corresponding to the cytokine binding module of IL-11Rα. Herein, an amino acid sequence which 'corresponds' to a reference region or sequence of a given peptide/polypeptide has at least 60%, e.g. one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of the reference region/sequence. The gp130, IL-11Rα and IL-11 may be from any species, and include isoforms, fragments, variants or homologues from any species.

In some embodiments a decoy receptor may be able to bind IL-11, e.g. with binding affinity of at least 100μM or less, optionally one of 10μM or less, 1μM or less, 100nM or less, or about 1 to 100nM. In some embodiments a decoy receptor may comprise all or part of the IL-11 binding domain and may optionally lack all or part of the transmembrane domains. The decoy receptor may optionally be fused to an immunoglobulin constant region, e.g. IgG Fc region.

In some embodiments an IL-11 binding agent may be provided in the form of a small molecule inhibitor of IL-11, e.g. IL-11 inhibitor described in Lay et al., Int. J. Oncol. (2012); 41(2): 759-764.

Agents that bind to an IL-11 receptor (IL-11R) may inhibit IL-11 mediated signalling by blocking the binding of IL-11 to an IL-11R or by preventing signal transduction via the gp130 co-receptors. Suitable IL-11R binding agents may be IL-11R inhibitors or IL-11R antagonists. In preferred embodiments the IL-11R is IL-11Rα and suitable binding agents may bind the IL-11Rα polypeptide and may be inhibitors or antagonists of IL-11Rα.

IL-11R binding agents, e.g. anti-IL-11R antibodies, according to the present invention may exhibit at least one of the following properties:

(a) Bind to human IL-11R with a K_D of 1μM or less, preferably one of ≤ 1μM, ≤ 100nM, ≤10nM, ≤1nM or ≤100pM;

(b) Inhibit IL-11R signalling, e.g. in a cell based assay in which the cells co-express IL-11Rα and gp130. Suitable cell based assays are ^{3}H-thymidine incorporation and Ba/F3 cell proliferation assays described in e.g. Curtis et al. Blood, 1997, 90(11) and Karpovich et al. Mol. Hum. Reprod. 2003 9(2): 75-80. For example, IC_{50} for an IL-11R binding agent may be determined by culturing Ba/F3 cells expressing IL-11Rα and gp130 in the presence of human IL-11 and the IL-11R binding agent, and measuring ^{3}H-thymidine incorporation into DNA. Suitable IL-11R binding agents may exhibit an IC_{50} of 10 μg/ml or less, preferably one of ≤ 5 μg/ml, ≤ 4 μg/ml, ≤ 3.5 μg/ml, ≤ 3 μg/ml, ≤ 2 μg/ml, ≤ 1 μg/ml, ≤ 0.9 μg/ml, ≤ 0.8 μg/ml, ≤ 0.7 μg/ml, ≤ 0.6 μg/ml, or ≤ 0.5 μg/ml in such an assay.

(c) Inhibit fibroblast proliferation, e.g. proliferation of cardiac/atrial fibroblasts. This can, for example, be evaluated in an assay wherein fibroblasts are stimulated with IL-11 or TGFβ1 and cell proliferation is monitored as described herein.

(d) Inhibit myofibroblast generation, e.g. from cardiac/atrial fibroblasts. This can, for example, be evaluated in an assay wherein fibroblasts are stimulated with IL-11 or TGFβ1 and myofibroblast generation is monitored, e.g. by measuring αSMA levels.

(e) Inhibit extracellular matrix production by fibroblasts, e.g. cardiac/atrial fibroblasts. This can, for example, be evaluated in an assay wherein fibroblasts are stimulated with IL-11 or TGFβ1 and production of extracellular matrix components is measured.

(f) Inhibit collagen and/or periostin gene or protein expression in fibroblasts, e.g. cardiac/atrial fibroblasts. This can, for example, be evaluated in an assay wherein fibroblasts are stimulated with IL-11 or TGFβ1 and collagen and/or periostin gene or protein expression is measured.

IL-11R binding agents may be of any kind, but in some embodiments an IL-11R binding agent may be an antibody, polypeptide, peptide, oligonucleotide, aptamer or small molecule.

Suitable anti-IL-11R antibodies will preferably bind to IL-11R (the antigen), preferably human IL-11R, and may have a dissociation constant (K_D) of one of ≤ 1μM, ≤ 100nM, ≤10nM, ≤1nM or ≤100pM. Binding affinity of an antibody for its target is often described in terms of its dissociation constant (K_D). Binding affinity can be measured by methods known in the art, such as by Surface Plasmon Resonance (SPR), or by a radiolabeled antigen binding assay (RIA) performed with the Fab version of the antibody and antigen molecule.

Anti-IL-11R antibodies may be antagonist antibodies that inhibit or reduce a biological activity of IL-11R. Anti-IL-11R antibodies may be antagonist antibodies that inhibit or reduce any function of IL-11R, in particular signalling. For example, antagonist IL-11R antibodies may inhibit or prevent binding of IL-11 to IL-11R, or may inhibit or prevent association of IL-11Rα with gp130 to form a functional receptor complex capable of productive signalling, e.g. in response to IL-11 binding.

Anti-IL-11R antibodies may be neutralising antibodies that neutralise the biological effect of IL-11R, e.g. its ability to initiate productive signalling mediated by binding of IL-11.

Neutralising activity may be measured by ability to neutralise IL-11 induced proliferation in the T11 mouse plasmacytoma cell line (Nordan, R. P. *et al*. (1987) J. Immunol. **139**:813).

Examples of known anti-IL-11R antibodies include monoclonal antibody clone 025 (Sino Biological), clone EPR5446 (Abcam), clone 473143 (R & D Systems), clones 8E2 and 8E4 described in US 2014/0219919 A1 and the monoclonal antibodies described in Blanc et al (J. Immunol Methods. 2000 Jul 31;241(1-2);43-59).

Peptide or polypeptide based IL-11R binding agents may be based on IL-11, e.g. mutant, variant or binding fragment of IL-11. Suitable peptide or polypeptide based agents may bind to IL-11R in a manner that does not lead to initiation of signal transduction or produces sub-optimal signaling. IL-11 mutants of this kind may act as competitive inhibitors of endogenous IL-11.

For example, W147A is an IL-11 antagonist in which the amino acid 147 is mutated from a tryptophan to an alanine, which destroys the so-called 'site III' of IL-11. This mutant can bind to the IL-11R, but engagement of the gp130 homodimer fails, resulting in efficient blockade of IL-11 signaling (Underhill-Day et al., 2003; Endocrinology 2003 Aug;144(8):3406-14). Lee et al (Am J respire Cell Mol Biol. 2008 Dec; 39(6):739-746) also report the generation of an IL-11 antagonist mutant (a "mutein") capable of specifically inhibiting the binding of IL-11 to IL-11Rα.

Menkhorst et al (Biology of Reproduction May 1, 2009 vol.80 no.5 920-927) describe a PEGylated IL-11 antagonist, PEGIL11A (CSL Limited, Parkvill, Victoria, Australia) which is effective to inhibit IL-11 action in female mice.

Pasqualini et al. Cancer (2015) 121(14):2411-2421 describe a ligand-directed, peptidomimetic drug, bone metastasis-targeting peptidomimetic-11 (BMTP-11) capable of binding to IL-11Rα.

In some embodiments an IL-11R binding agent may be provided in the form of a small molecule inhibitor of IL-11R.

The inventors have identified that upregulation of IL-11 expression is consistent with the molecular mechanism of fibrosis and that inhibition of IL-11 activity leads to a reduction in the molecular basis for fibrosis. Accordingly, in some aspects of the present invention

treatment, prevention or alleviation of fibrosis may be provided by administration of an agent capable of preventing or reducing the expression of IL-11 by cells of the subject, e.g. by fibroblasts or myofibroblasts.

Suitable agents may be of any kind, but in some embodiments an agent capable of preventing or reducing the expression of IL-11 may be a small molecule or an oligonucleotide.

Taki et al (Clin Exp Immunol. 1998 Apr; 112(1): 133-138) report a reduction in the expression of IL-11 in rheumatoid synovial cells upon treatment with indomethacin, dexamethasone or interferon-gamma (IFNγ).

In some embodiments an agent capable of preventing or reducing the expression of IL-11 may be an oligonucleotide capable of repressing or silencing expression of IL-11.

Accordingly, the present invention also includes the use of techniques known in the art for the therapeutic down regulation of IL-11 expression. These include the use of antisense oligonucleotides and RNA interference (RNAi). As in other aspects of the present invention, these techniques may be used in the treatment of fibrosis.

Accordingly, in one aspect of the present invention a method of treating or preventing fibrosis is provided, the method comprising administering to a subject in need of treatment a therapeutically effective amount of an agent capable of preventing or reducing the expression of IL-11, wherein the agent comprises a vector comprising a therapeutic oligonucleotide capable of repressing or silencing expression of IL-11.

In another aspect of the present invention a method of treating or preventing fibrosis is provided, the method comprising administering to a subject in need of treatment a therapeutically effective amount of an agent capable of preventing or reducing the expression of IL-11, wherein the agent comprises an oligonucleotide vector, optionally a viral vector, encoding a therapeutic oligonucleotide capable of being expressed in cells of the subject, the expressed therapeutic oligonucleotide being capable of repressing or silencing expression of IL-11.

The ability of an agent to prevent or reduce the expression of IL-11 may be assayed by determining the ability of the agent to inhibit IL-11 gene or protein expression by fibroblasts or myofibroblasts, e.g. cardiac/atrial fibroblasts or myofibroblasts. This can, for example, be evaluated in an assay wherein fibroblasts or myofibroblasts are stimulated with IL-11 or TGFβ1, and IL-11 gene or protein expression is measured.

Reducing the amount of IL-11R available for binding to IL-11 and initiation of productive signalling provides an alternative means of reducing the level of IL-11 stimulated signalling. Accordingly, in related aspects of the present invention, treatment, prevention or alleviation of fibrosis may be provided by administration of an agent capable of preventing or reducing the expression of IL-11R by cells of the subject, e.g. by fibroblasts or myofibroblasts.

In some embodiments an agent capable of preventing or reducing the expression of IL-11R may be an oligonucleotide capable of repressing or silencing expression of IL-11R.

Accordingly, the present invention also includes the use of techniques known in the art for the therapeutic down regulation of IL-11R expression. These include the use of antisense oligonucleotides and RNA interference (RNAi). As in other aspects of the present invention, these techniques may be used in the treatment of fibrosis.

Accordingly, in one aspect of the present invention a method of treating or preventing fibrosis is provided, the method comprising administering to a subject in need of treatment a therapeutically effective amount of an agent capable of preventing or reducing the expression of IL-11R, wherein the agent comprises a vector comprising a therapeutic oligonucleotide capable of repressing or silencing expression of IL-11R.

In another aspect of the present invention a method of treating or preventing fibrosis is provided, the method comprising administering to a subject in need of treatment a therapeutically effective amount of an agent capable of preventing or reducing the expression of IL-11R, wherein the agent comprises an oligonucleotide vector, optionally a viral vector, encoding a therapeutic oligonucleotide capable of being expressed in cells of the subject, the expressed therapeutic oligonucleotide being capable of repressing or silencing expression of IL-11R.

The ability of an agent to prevent or reduce the expression of IL-11R may be assayed by determining the ability of the agent to inhibit IL-11R gene or protein expression by fibroblasts or myofibroblasts, e.g. cardiac/atrial fibroblasts or myofibroblasts. This can, for example, be evaluated in an assay wherein fibroblasts or myofibroblasts are stimulated with IL-11 or TGFβ1, and IL-11R gene or protein expression is measured.

In preferred embodiments, the IL-11R may be IL-11Rα.

Antibodies

In this specification "antibody" includes a fragment or derivative of an antibody, or a synthetic antibody or synthetic antibody fragment.

Antibodies may be provided in isolated or purified form. Antibodies may be formulated as a pharmaceutical composition or medicament.

In view of today's techniques in relation to monoclonal antibody technology, antibodies can be prepared to most antigens. The antigen-binding portion may be a part of an antibody (for example a Fab fragment) or a synthetic antibody fragment (for example a single chain Fv fragment [ScFv]). Suitable monoclonal antibodies to selected antigens may be prepared by known techniques, for example those disclosed in "Monoclonal Antibodies: A manual of techniques ", H Zola (CRC Press, 1988) and in "Monoclonal Hybridoma Antibodies: Techniques and Applications ", J G R Hurrell (CRC Press, 1982). Chimaeric antibodies are discussed by Neuberger et al (1988, 8th International Biotechnology Symposium Part 2, 792-799).

Monoclonal antibodies (mAbs) are useful in the methods of the invention and are a homogenous population of antibodies specifically targeting a single epitope on an antigen.

Polyclonal antibodies are useful in the methods of the invention. Monospecific polyclonal antibodies are preferred. Suitable polyclonal antibodies can be prepared using methods well known in the art.

Antigen binding fragments of antibodies, such as Fab and Fab_2 fragments may also be used/provided as can genetically engineered antibodies and antibody fragments. The

variable heavy (V_H) and variable light (V_L) domains of the antibody are involved in antigen recognition, a fact first recognised by early protease digestion experiments. Further confirmation was found by "humanisation" of rodent antibodies. Variable domains of rodent origin may be fused to constant domains of human origin such that the resultant antibody retains the antigenic specificity of the rodent parented antibody (Morrison et al (1984) Proc. Natl. Acad. Sd. USA 81, 6851-6855).

That antigenic specificity is conferred by variable domains and is independent of the constant domains is known from experiments involving the bacterial expression of antibody fragments, all containing one or more variable domains. These molecules include Fab-like molecules (Better et al (1988) Science 240, 1041); Fv molecules (Skerra et al (1988) Science 240, 1038); single-chain Fv (ScFv) molecules where the V_H and V_L partner domains are linked via a flexible oligopeptide (Bird et al (1988) Science 242, 423; Huston et al (1988) Proc. Natl. Acad. Sd. USA 85, 5879) and single domain antibodies (dAbs) comprising isolated V domains (Ward et al (1989) Nature 341, 544). A general review of the techniques involved in the synthesis of antibody fragments which retain their specific binding sites is to be found in Winter & Milstein (1991) Nature 349, 293- 299.

By "ScFv molecules" we mean molecules wherein the V_H and V_L partner domains are covalently linked, e.g. by a flexible oligopeptide.

Fab, Fv, ScFv and dAb antibody fragments can all be expressed in and secreted from E. coli, thus allowing the facile production of large amounts of the said fragments.

Whole antibodies, and $F(ab')_2$ fragments are "bivalent". By "bivalent" we mean that the said antibodies and $F(ab')_2$ fragments have two antigen combining sites. In contrast, Fab, Fv, ScFv and dAb fragments are monovalent, having only one antigen combining site. Synthetic antibodies which bind to IL-11 or IL-11R may also be made using phage display technology as is well known in the art.

Antibodies may be produced by a process of affinity maturation in which a modified antibody is generated that has an improvement in the affinity of the antibody for antigen, compared to an unmodified parent antibody. Affinity-matured antibodies may be produced by procedures known in the art, e.g., Marks *et al.,Rio/Technology* 10:779-783 (1992); Barbas *et al. Proc Nat. Acad. Sci. USA* 91:3809-3813 (1994); Schier *et al. Gene* 169:147-

155 (1995); Yelton *et al. J. Immunol.* 155:1994-2004 (1995); Jackson *et al., J. Immunol.* 154(7):331 0-15 9 (1995); and Hawkins *et al, J. Mol. Biol.* 226:889-896 (1992).

Antibodies according to the present invention preferably exhibit specific binding to IL-11 or IL-11R. An antibody that specifically binds to a target molecule preferably binds the target with greater affinity, and/or with greater duration than it binds to other targets. In one embodiment, the extent of binding of an antibody to an unrelated target is less than about 10% of the binding of the antibody to the target as measured, e.g., by ELISA, or by a radioimmunoassay (RIA). Alternatively, the binding specificity may be reflected in terms of binding affinity where the antibody binds to IL-11 or IL-11R with a K_D that is at least 0.1 order of magnitude (i.e. 0.1 x 10^n, where n is an integer representing the order of magnitude) greater than the K_D of the antibody towards another target molecule, e.g. another member of the IL-11 family such as IL-6 or the IL-6 receptor. This may optionally be one of at least 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, or 2.0.

Antibodies may be detectably labelled or, at least, capable of detection. Such antibodies being useful for both *in vivo* (e.g. imaging methods) and *in vitro* (e.g. assay methods) applications For example, the antibody may be labelled with a radioactive atom or a coloured molecule or a fluorescent molecule or a molecule which can be readily detected in any other way. Suitable detectable molecules include fluorescent proteins, luciferase, enzyme substrates, and radiolabels. The binding moiety may be directly labelled with a detectable label or it may be indirectly labelled. For example, the binding moiety may be an unlabelled antibody which can be detected by another antibody which is itself labelled. Alternatively, the second antibody may have bound to it biotin and binding of labelled streptavidin to the biotin is used to indirectly label the first antibody.

Aspects of the present invention include bi-specific antibodies, e.g. composed of two different fragments of two different antibodies, such that the bi-specific antibody binds two types of antigen. One of the antigens is IL-11 or IL-11R, the bi-specific antibody comprising a fragment as described herein that binds to IL-11 or IL-11R. The antibody may contain a different fragment having affinity for a second antigen, which may be any desired antigen. Techniques for the preparation of bi-specific antibodies are well known in the art, e.g. see Mueller, D et al., (2010 *Biodrugs* **24** (2): 89–98), Wozniak-Knopp G et al., (2010 *Protein Eng Des* **23** (4): 289–297. Baeuerle, PA et al., (2009 *Cancer Res* **69** (12): 4941–4944).

In some embodiments, the bispecific antibody is provided as a fusion protein of two single-chain variable fragments (scFV) format, comprising a V_H and V_L of a IL-11 or IL-11R binding antibody or antibody fragment, and a V_H and V_L of an another antibody or antibody fragment.

Bispecific antibodies and bispecific antigen binding fragments may be provided in any suitable format, such as those formats described in Kontermann MAbs 2012, 4(2): 182-197, which is hereby incorporated by reference in its entirety. For example, a bispecific antibody or bispecific antigen binding fragment may be a bispecific antibody conjugate (e.g. an IgG2, F(ab')$_2$ or CovX-Body), a bispecific IgG or IgG-like molecule (e.g. an IgG, scFv$_4$-Ig, IgG-scFv, scFv-IgG, DVD-Ig, IgG-sVD, sVD-IgG, 2 in 1-IgG, mAb^2, or Tandemab common LC), an asymmetric bispecific IgG or IgG-like molecule (e.g. a kih IgG, kih IgG common LC, CrossMab, kih IgG-scFab, mAb-Fv, charge pair or SEED-body), a small bispecific antibody molecule (e.g. a Diabody (Db), dsDb, DART, scDb, tandAbs, tandem scFv (taFv), tandem dAb/VHH, triple body, triple head, Fab-scFv, or F(ab')$_2$-scFv$_2$), a bispecific Fc and C_H3 fusion protein (e.g. a taFv-Fc, Di-diabody, scDb-C_H3, scFv-Fc-scFv, HCAb-VHH, scFv-kih-Fc, or scFv-kih-C_H3), or a bispecific fusion protein (e.g. a scFv$_2$-albumin, scDb-albumin, taFv-toxin, DNL-Fab$_3$, DNL-Fab$_4$-IgG, DNL-Fab$_4$-IgG-cytokine$_2$). See in particular Figure 2 of Kontermann MAbs 2012, 4(2): 182-19.

Methods for producing bispecific antibodies include chemically crosslinking antibodies or antibody fragments, e.g. with reducible disulphide or non-reducible thioether bonds, for example as described in Segal and Bast, 2001. Production of Bispecific Antibodies. Current Protocols in Immunology. 14:IV:2.13:2.13.1–2.13.16, which is hereby incorporated by reference in its entirety. For example, *N*-succinimidyl-3-(-2-pyridyldithio)-propionate (SPDP) can be used to chemically crosslink e.g. Fab fragments via hinge region SH- groups, to create disulfide-linked bispecific F(ab)$_2$ heterodimers.

Other methods for producing bispecific antibodies include fusing antibody-producing hybridomas e.g. with polyethylene glycol, to produce a quadroma cell capable of secreting bispecific antibody, for example as described in D. M. and Bast, B. J. 2001. Production of Bispecific Antibodies. Current Protocols in Immunology. 14:IV:2.13:2.13.1–2.13.16.

Bispecific antibodies and bispecific antigen binding fragments can also be produced recombinantly, by expression from e.g. a nucleic acid construct encoding polypeptides for

the antigen binding molecules, for example as described in Antibody Engineering: Methods and Protocols, Second Edition (Humana Press, 2012), at Chapter 40: Production of Bispecific Antibodies: Diabodies and Tandem scFv (Hornig and Färber-Schwarz), or French, How to make bispecific antibodies, Methods Mol. Med. 2000; 40:333-339.

For example, a DNA construct encoding the light and heavy chain variable domains for the two antigen binding domains (i.e. the light and heavy chain variable domains for the antigen binding domain capable of binding IL-11 or IL-11R, and the light and heavy chain variable domains for the antigen binding domain capable of binding to another target protein), and including sequences encoding a suitable linker or dimerization domain between the antigen binding domains can be prepared by molecular cloning techniques. Recombinant bispecific antibody can thereafter be produced by expression (e.g. *in vitro*) of the construct in a suitable host cell (e.g. a mammalian host cell), and expressed recombinant bispecific antibody can then optionally be purified.

Aptamers

Aptamers, also called nucleic acid ligands, are nucleic acid molecules characterised by the ability to bind to a target molecule with high specificity and high affinity. Almost every aptamer identified to date is a non-naturally occurring molecule.

Aptamers to a given target (e.g. IL-11 or IL-11R) may be identified and/or produced by the method of Systematic Evolution of Ligands by EXponential enrichment (SELEX™). Aptamers and SELEX are described in Tuerk and Gold (Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science. 1990 Aug 3;249(4968):505-10) and in WO91/19813.

Aptamers may be DNA or RNA molecules and may be single stranded or double stranded. The aptamer may comprise chemically modified nucleic acids, for example in which the sugar and/or phosphate and/or base is chemically modified. Such modifications may improve the stability of the aptamer or make the aptamer more resistant to degradation and may include modification at the 2′ position of ribose.

Aptamers may be synthesised by methods which are well known to the skilled person. For example, aptamers may be chemically synthesised, e.g. on a solid support.

Solid phase synthesis may use phosphoramidite chemistry. Briefly, a solid supported nucleotide is detritylated, then coupled with a suitably activated nucleoside phosphoramidite to form a phosphite triester linkage. Capping may then occur, followed by oxidation of the phosphite triester with an oxidant, typically iodine. The cycle may then be repeated to assemble the aptamer.

Aptamers can be thought of as the nucleic acid equivalent of monoclonal antibodies and often have K_d's in the nM or pM range, e.g. less than one of 500nM, 100nM, 50nM, 10nM, 1nM, 500pM, 100pM. As with monoclonal antibodies, they may be useful in virtually any situation in which target binding is required, including use in therapeutic and diagnostic applications, *in vitro* or *in vivo*. *In vitro* diagnostic applications may include use in detecting the presence or absence of a target molecule.

Aptamers according to the present invention may be provided in purified or isolated form. Aptamers according to the present invention may be formulated as a pharmaceutical composition or medicament.

Suitable aptamers may optionally have a minimum length of one of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 nucleotides

Suitable aptamers may optionally have a maximum length of one of 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 nucleotides

Suitable aptamers may optionally have a length of one of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 nucleotides.

<u>Oligonucleotide repression of IL-11 or IL-11R expression</u>

Oligonucleotide molecules, particularly RNA, may be employed to regulate gene expression. These include antisense oligonucleotides, targeted degradation of mRNAs by small interfering RNAs (siRNAs), post transcriptional gene silencing (PTGs),

developmentally regulated sequence-specific translational repression of mRNA by micro-RNAs (miRNAs) and targeted transcriptional gene silencing.

An antisense oligonucleotide is an oligonucleotide, preferably single stranded, that targets and binds, by complementary sequence binding, to a target oligonucleotide, e.g. mRNA. Where the target oligonucleotide is an mRNA, binding of the antisense to the mRNA blocks translation of the mRNA and expression of the gene product. Antisense oligonucleotides may be designed to bind sense genomic nucleic acid and inhibit transcription of a target nucleotide sequence.

In view of the known nucleic acid sequences for IL-11 (e.g. the known mRNA sequences available from GenBank® under accession no.s: BC012506.1 GI:15341754 (human), BC134354.1 GI:126632002 (mouse), AF347935.1 GI:13549072 (rat)) and IL-11R (e.g. the known mRNA sequences available from GenBank® under accession no.s: NM_001142784.2 GI:391353394 (human), NM_001163401.1 GI:254281268 (mouse), NM_139116.1 GI:20806172 (rat)), oligonucleotides may be designed to repress or silence the expression of IL-11 or IL-11R. Such oligonucleotides may have any length, but may preferably be short, e.g. less than 100 nucleotides, e.g. 10-40 nucleotides, or 20-50 nucleotides, and may comprise a nucleotide sequence having complete- or near-complementarity (e.g. 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% complementarity) to a sequence of nucleotides of corresponding length in the target oligonucleotide, e.g. the IL-11 or IL-11R mRNA. The complementary region of the nucleotide sequence may have any length, but is preferably at least 5, and optionally no more than 50, nucleotides long, e.g. one of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides.

Repression of IL-11 or IL-11R expression will preferably result in a decrease in the quantity of IL-11 or IL-11R expressed by a cell, e.g. by a fibroblast or myofibroblast. For example, in a given cell the repression of IL-11 or IL-11R by administration of a suitable nucleic acid will result in a decrease in the quantity of IL-11 or IL-11R expressed by that cell relative to an untreated cell. Repression may be partial. Preferred degrees of repression are at least 50%, more preferably one of at least 60%, 70%, 80%, 85% or 90%. A level of repression between 90% and 100% is considered a 'silencing' of expression or function.

A role for the RNAi machinery and small RNAs in targeting of heterochromatin complexes and epigenetic gene silencing at specific chromosomal loci has been demonstrated. Double-stranded RNA (dsRNA)-dependent post transcriptional silencing, also known as RNA interference (RNAi), is a phenomenon in which dsRNA complexes can target specific genes of homology for silencing in a short period of time. It acts as a signal to promote degradation of mRNA with sequence identity. A 20-nt siRNA is generally long enough to induce gene-specific silencing, but short enough to evade host response. The decrease in expression of targeted gene products can be extensive with 90% silencing induced by a few molecules of siRNA. RNAi based therapeutics have been progressed into Phase I, II and III clinical trials for a number of indications (Nature 2009 Jan 22; 457(7228):426-433).

In the art, these RNA sequences are termed "short or small interfering RNAs" (siRNAs) or "microRNAs" (miRNAs) depending on their origin. Both types of sequence may be used to down-regulate gene expression by binding to complementary RNAs and either triggering mRNA elimination (RNAi) or arresting mRNA translation into protein. siRNA are derived by processing of long double stranded RNAs and when found in nature are typically of exogenous origin. Micro-interfering RNAs (miRNA) are endogenously encoded small non-coding RNAs, derived by processing of short hairpins. Both siRNA and miRNA can inhibit the translation of mRNAs bearing partially complimentary target sequences without RNA cleavage and degrade mRNAs bearing fully complementary sequences.

Accordingly, the present invention provides the use of oligonucleotide sequences for down-regulating the expression of IL-11 or IL-11R.

siRNA ligands are typically double stranded and, in order to optimise the effectiveness of RNA mediated down-regulation of the function of a target gene, it is preferred that the length of the siRNA molecule is chosen to ensure correct recognition of the siRNA by the RISC complex that mediates the recognition by the siRNA of the mRNA target and so that the siRNA is short enough to reduce a host response.

miRNA ligands are typically single stranded and have regions that are partially complementary enabling the ligands to form a hairpin. miRNAs are RNA genes which are transcribed from DNA, but are not translated into protein. A DNA sequence that codes for a miRNA gene is longer than the miRNA. This DNA sequence includes the miRNA

sequence and an approximate reverse complement. When this DNA sequence is transcribed into a single-stranded RNA molecule, the miRNA sequence and its reverse-complement base pair to form a partially double stranded RNA segment. The design of microRNA sequences is discussed in John et al, PLoS Biology, 11(2), 1862-1879, 2004.

Typically, the RNA ligands intended to mimic the effects of siRNA or miRNA have between 10 and 40 ribonucleotides (or synthetic analogues thereof), more preferably between 17 and 30 ribonucleotides, more preferably between 19 and 25 ribonucleotides and most preferably between 21 and 23 ribonucleotides. In some embodiments of the invention employing double-stranded siRNA, the molecule may have symmetric 3' overhangs, e.g. of one or two (ribo)nucleotides, typically a UU of dTdT 3' overhang. Based on the disclosure provided herein, the skilled person can readily design suitable siRNA and miRNA sequences, for example using resources such the Ambion siRNA finder. siRNA and miRNA sequences can be synthetically produced and added exogenously to cause gene downregulation or produced using expression systems (e.g. vectors). In a preferred embodiment the siRNA is synthesized synthetically.

Longer double stranded RNAs may be processed in the cell to produce siRNAs (see for example Myers (2003) *Nature Biotechnology 21:324-328*). The longer dsRNA molecule may have symmetric 3' or 5' overhangs, e.g. of one or two (ribo)nucleotides, or may have blunt ends. The longer dsRNA molecules may be 25 nucleotides or longer. Preferably, the longer dsRNA molecules are between 25 and 30 nucleotides long. More preferably, the longer dsRNA molecules are between 25 and 27 nucleotides long. Most preferably, the longer dsRNA molecules are 27 nucleotides in length. dsRNAs 30 nucleotides or more in length may be expressed using the vector pDECAP (Shinagawa et al., Genes and Dev., 17, 1340-5, 2003).

Another alternative is the expression of a short hairpin RNA molecule (shRNA) in the cell. shRNAs are more stable than synthetic siRNAs. A shRNA consists of short inverted repeats separated by a small loop sequence. One inverted repeat is complimentary to the gene target. In the cell the shRNA is processed by DICER into a siRNA which degrades the target gene mRNA and suppresses expression. In a preferred embodiment the shRNA is produced endogenously (within a cell) by transcription from a vector. shRNAs may be produced within a cell by transfecting the cell with a vector encoding the shRNA sequence under control of a RNA polymerase III promoter such as the human H1 or 7SK promoter or a RNA polymerase II promoter. Alternatively, the shRNA may be

synthesised exogenously (*in vitro*) by transcription from a vector. The shRNA may then be introduced directly into the cell. Preferably, the shRNA molecule comprises a partial sequence of IL-11 or IL-11R. Preferably, the shRNA sequence is between 40 and 100 bases in length, more preferably between 40 and 70 bases in length. The stem of the hairpin is preferably between 19 and 30 base pairs in length. The stem may contain G-U pairings to stabilise the hairpin structure.

siRNA molecules, longer dsRNA molecules or miRNA molecules may be made recombinantly by transcription of a nucleic acid sequence, preferably contained within a vector. Preferably, the siRNA molecule, longer dsRNA molecule or miRNA molecule comprises a partial sequence of IL-11 or IL-11R.

In one embodiment, the siRNA, longer dsRNA or miRNA is produced endogenously (within a cell) by transcription from a vector. The vector may be introduced into the cell in any of the ways known in the art. Optionally, expression of the RNA sequence can be regulated using a tissue specific (e.g. heart, liver, kidney or eye specific) promoter. In a further embodiment, the siRNA, longer dsRNA or miRNA is produced exogenously (*in vitro*) by transcription from a vector.

Suitable vectors may be oligonucleotide vectors configured to express the oligonucleotide agent capable of IL-11 or IL-11R repression. Such vectors may be viral vectors or plasmid vectors. The therapeutic oligonucleotide may be incorporated in the genome of a viral vector and be operably linked to a regulatory sequence, e.g. promoter, which drives its expression. The term “operably linked” may include the situation where a selected nucleotide sequence and regulatory nucleotide sequence are covalently linked in such a way as to place the expression of a nucleotide sequence under the influence or control of the regulatory sequence. Thus a regulatory sequence is operably linked to a selected nucleotide sequence if the regulatory sequence is capable of effecting transcription of a nucleotide sequence which forms part or all of the selected nucleotide sequence.

Viral vectors encoding promoter-expressed siRNA sequences are known in the art and have the benefit of long term expression of the therapeutic oligonucleotide. Examples include lentiviral (Nature 2009 Jan 22; 457(7228):426-433), adenovirus (Shen et al., FEBS Lett 2003 Mar 27;539(1-3)111-4) and retroviruses (Barton and Medzhitov PNAS November 12, 2002 vol.99, no.23 14943-14945).

In other embodiments a vector may be configured to assist delivery of the therapeutic oligonucleotide to the site at which repression of IL-11 or IL-11R expression is required. Such vectors typically involve complexing the oligonucleotide with a positively charged vector (e.g., cationic cell penetrating peptides, cationic polymers and dendrimers, and cationic lipids); conjugating the oligonucleotide with small molecules (e.g., cholesterol, bile acids, and lipids), polymers, antibodies, and RNAs; or encapsulating the oligonucleotide in nanoparticulate formulations (Wang et al., AAPS J. 2010 Dec; 12(4): 492-503).

In one embodiment, a vector may comprise a nucleic acid sequence in both the sense and antisense orientation, such that when expressed as RNA the sense and antisense sections will associate to form a double stranded RNA.

Alternatively, siRNA molecules may be synthesized using standard solid or solution phase synthesis techniques which are known in the art. Linkages between nucleotides may be phosphodiester bonds or alternatives, for example, linking groups of the formula P(O)S, (thioate); P(S)S, (dithioate); P(O)NR'2; P(O)R'; P(O)OR6; CO; or CONR'2 wherein R is H (or a salt) or alkyl (1-12C) and R6 is alkyl (1-9C) is joined to adjacent nucleotides through-O-or-S-.

Modified nucleotide bases can be used in addition to the naturally occurring bases, and may confer advantageous properties on siRNA molecules containing them.

For example, modified bases may increase the stability of the siRNA molecule, thereby reducing the amount required for silencing. The provision of modified bases may also provide siRNA molecules which are more, or less, stable than unmodified siRNA.

The term ‘modified nucleotide base’ encompasses nucleotides with a covalently modified base and/or sugar. For example, modified nucleotides include nucleotides having sugars which are covalently attached to low molecular weight organic groups other than a hydroxyl group at the 3'position and other than a phosphate group at the 5'position. Thus modified nucleotides may also include 2'substituted sugars such as 2'-O-methyl- ; 2'-O-alkyl ; 2'-O-allyl ; 2'-S-alkyl; 2'-S-allyl; 2'-fluoro- ; 2'-halo or azido-ribose, carbocyclic sugar analogues, a-anomeric sugars; epimeric sugars such as arabinose, xyloses or lyxoses,

pyranose sugars, furanose sugars, and sedoheptulose.

Modified nucleotides are known in the art and include alkylated purines and pyrimidines, acylated purines and pyrimidines, and other heterocycles. These classes of pyrimidines and purines are known in the art and include pseudoisocytosine, N4,N4-ethanocytosine, 8-hydroxy-N6-methyladenine, 4-acetylcytosine,5-(carboxyhydroxylmethyl) uracil, 5 fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxymethylaminomethyl uracil, dihydrouracil, inosine, N6-isopentyl-adenine, 1-methyladenine, 1-methylpseudouracil, 1-methylguanine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyl uracil, 5-methoxy amino methyl-2-thiouracil, -D-mannosylqueosine, 5-methoxycarbonylmethyluracil, 5methoxyuracil, 2 methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methyl ester, psueouracil, 2-thiocytosine, 5-methyl-2 thiouracil, 2-thiouracil, 4-thiouracil, 5methyluracil, N-uracil-5-oxyacetic acid methylester, uracil 5-oxyacetic acid, queosine, 2-thiocytosine, 5-propyluracil, 5-propylcytosine, 5-ethyluracil, 5ethylcytosine, 5-butyluracil, 5-pentyluracil, 5-pentylcytosine, and 2,6,diaminopurine, methylpsuedouracil, 1-methylguanine, 1-methylcytosine.

Methods relating to the use of RNAi to silence genes in C. elegans, Drosophila, plants, and mammals are known in the art (Fire A, et al., 1998 Nature 391:806-811; Fire, A. Trends Genet. 15, 358-363 (1999); Sharp, P. A. RNA interference 2001. Genes Dev. 15, 485-490 (2001); Hammond, S. M., et al., Nature Rev. Genet. 2, 110-1119 (2001); Tuschl, T. Chem. Biochem. 2, 239-245 (2001); Hamilton, A. et al., Science 286, 950-952 (1999); Hammond, S. M., et al., Nature 404, 293-296 (2000); Zamore, P. D., et al., Cell 101, 25-33 (2000); Bernstein, E., et al., Nature 409, 363-366 (2001); Elbashir, S. M., et al., Genes Dev. 15, 188-200 (2001); WO0129058; WO9932619, and Elbashir S M, et al., 2001 Nature 411:494-498).

Accordingly, the invention provides nucleic acid that is capable, when suitably introduced into or expressed within a mammalian, e.g. human, cell that otherwise expresses IL-11 or IL-11R, of suppressing IL-11 or IL-11R expression by RNAi.

The nucleic acid may have substantial sequence identity to a portion of IL-11 or IL-11R mRNA, as defined in GenBank accession no. NM_000641.3 GI:391353405 (IL-11) or U32324.1 GI:975336 (IL-11R), or the complementary sequence to said mRNA.

The nucleic acid may be a double-stranded siRNA. (As the skilled person will appreciate, and as explained further below, a siRNA molecule may include a short 3' DNA sequence also.)

Alternatively, the nucleic acid may be a DNA (usually double-stranded DNA) which, when transcribed in a mammalian cell, yields an RNA having two complementary portions joined via a spacer, such that the RNA takes the form of a hairpin when the complementary portions hybridise with each other. In a mammalian cell, the hairpin structure may be cleaved from the molecule by the enzyme DICER, to yield two distinct, but hybridised, RNA molecules.

In some preferred embodiments, the nucleic acid is generally targeted to the sequence of one of SEQ ID NOs 2 to 5 (IL-11; Figure 11) or to one of SEQ ID NOs 7 to 10 (IL-11R; Figure 12).

Only single-stranded (i.e. non self-hybridised) regions of an mRNA transcript are expected to be suitable targets for RNAi. It is therefore proposed that other sequences very close in the IL-11 or IL-11R mRNA transcript to the sequence represented by one of SEQ ID NOs 2 to 5 or 7 to 10 may also be suitable targets for RNAi. Such target sequences are preferably 17-23 nucleotides in length and preferably overlap one of SEQ ID NOs 2 to 5 or 7 to 10 by at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 or all 19 nucleotides (at either end of one of SEQ ID NOs 2 to 5 or 7 to 10).

Accordingly, the invention provides nucleic acid that is capable, when suitably introduced into or expressed within a mammalian cell that otherwise expresses IL-11 or IL-11R, of suppressing IL-11 or IL-11R expression by RNAi, wherein the nucleic acid is generally targeted to the sequence of one of SEQ ID NOs 2 to 5 or 7 to 10.

By "generally targeted" the nucleic acid may target a sequence that overlaps with SEQ ID NOs 2 to 5 or 7 to 10. In particular, the nucleic acid may target a sequence in the mRNA of human IL-11 or IL-11R that is slightly longer or shorter than one of SEQ ID NOs 2 to 5 or 7 to 10 (preferably from 17-23 nucleotides in length), but is otherwise identical to one of SEQ ID NOs 2 to 5 or 7 to 10.

It is expected that perfect identity/complementarity between the nucleic acid of the invention and the target sequence, although preferred, is not essential. Accordingly, the nucleic acid of the invention may include a single mismatch compared to the mRNA of IL-11 or IL-11R. It is expected, however, that the presence of even a single mismatch is likely to lead to reduced efficiency, so the absence of mismatches is preferred. When present, 3' overhangs may be excluded from the consideration of the number of mismatches.

The term "complementarity" is not limited to conventional base pairing between nucleic acid consisting of naturally occurring ribo- and/or deoxyribonucleotides, but also includes base pairing between mRNA and nucleic acids of the invention that include non-natural nucleotides.

In one embodiment, the nucleic acid (herein referred to as double-stranded siRNA) includes the double-stranded RNA sequences shown in Figure 13 (IL-11; SEQ ID NOs 11 to 14).

In another embodiment, the nucleic acid (herein referred to as double-stranded siRNA) includes the double-stranded RNA sequences shown in Figure 14 (IL-11R; SEQ ID NOs 15 to 18).

However, it is also expected that slightly shorter or longer sequences directed to the same region of IL-11 or IL-11R mRNA will also be effective. In particular, it is expected that double-stranded sequences between 17 and 23 bp in length will also be effective.

The strands that form the double-stranded RNA may have short 3' dinucleotide overhangs, which may be DNA or RNA. The use of a 3' DNA overhang has no effect on siRNA activity compared to a 3' RNA overhang, but reduces the cost of chemical synthesis of the nucleic acid strands (Elbashir et al., 2001c). For this reason, DNA dinucleotides may be preferred.

When present, the dinucleotide overhangs may be symmetrical to each other, though this is not essential. Indeed, the 3' overhang of the sense (upper) strand is irrelevant for RNAi activity, as it does not participate in mRNA recognition and degradation (Elbashir et al., 2001a, 2001b, 2001c).

While RNAi experiments in Drosophila show that antisense 3' overhangs may participate in mRNA recognition and targeting (Elbashir et al. 2001c), 3' overhangs do not appear to be necessary for RNAi activity of siRNA in mammalian cells. Incorrect annealing of 3' overhangs is therefore thought to have little effect in mammalian cells (Elbashir et al. 2001c; Czauderna et al. 2003).

Any dinucleotide overhang may therefore be used in the antisense strand of the siRNA. Nevertheless, the dinucleotide is preferably -UU or –UG (or –TT or –TG if the overhang is DNA), more preferably -UU (or –TT). The –UU (or –TT) dinucleotide overhang is most effective and is consistent with (i.e. capable of forming part of) the RNA polymerase III end of transcription signal (the terminator signal is TTTTT). Accordingly, this dinucleotide is most preferred. The dinucleotides AA, CC and GG may also be used, but are less effective and consequently less preferred.

Moreover, the 3' overhangs may be omitted entirely from the siRNA.

The invention also provides single-stranded nucleic acids (herein referred to as single-stranded siRNAs) respectively consisting of a component strand of one of the aforementioned double-stranded nucleic acids, preferably with the 3'-overhangs, but optionally without. The invention also provides kits containing pairs of such single-stranded nucleic acids, which are capable of hybridising with each other in vitro to form the aforementioned double-stranded siRNAs, which may then be introduced into cells.

The invention also provides DNA that, when transcribed in a mammalian cell, yields an RNA (herein also referred to as an shRNA) having two complementary portions which are capable of self-hybridising to produce a double-stranded motif, e.g. including a sequence selected from the group consisting of SEQ ID No.s 11 to 14 or 15 to 18 or a sequence that differs from any one of the aforementioned sequences by a single base pair substitution.

The complementary portions will generally be joined by a spacer, which has suitable length and sequence to allow the two complementary portions to hybridise with each other. The two complementary (i.e. sense and antisense) portions may be joined 5'-3' in either order. The spacer will typically be a short sequence, of approximately 4-12 nucleotides, preferably 4-9 nucleotides, more preferably 6-9 nucleotides.

Preferably the 5' end of the spacer (immediately 3' of the upstream complementary portion) consists of the nucleotides –UU- or –UG-, again preferably –UU- (though, again, the use of these particular dinucleotides is not essential). A suitable spacer, recommended for use in the pSuper system of OligoEngine (Seattle, Washington, USA) is UUCAAGAGA. In this and other cases, the ends of the spacer may hybridise with each other, e.g. elongating the double-stranded motif beyond the exact sequences of SEQ ID NOs 11 to 14 or 15 to 18 by a small number (e.g. 1 or 2) of base pairs.

Similarly, the transcribed RNA preferably includes a 3' overhang from the downstream complementary portion. Again, this is preferably –UU or –UG, more preferably –UU.

Such shRNA molecules may then be cleaved in the mammalian cell by the enzyme DICER to yield a double-stranded siRNA as described above, in which one or each strand of the hybridised dsRNA includes a 3' overhang.

Techniques for the synthesis of the nucleic acids of the invention are of course well known in the art.

The skilled person is well able to construct suitable transcription vectors for the DNA of the invention using well-known techniques and commercially available materials. In particular, the DNA will be associated with control sequences, including a promoter and a transcription termination sequence.

Of particular suitability are the commercially available pSuper and pSuperior systems of OligoEngine (Seattle, Washington, USA). These use a polymerase-III promoter (H1) and a T_5 transcription terminator sequence that contributes two U residues at the 3' end of the transcript (which, after DICER processing, provide a 3' UU overhang of one strand of the siRNA).

Another suitable system is described in Shin et al. (RNA, 2009 May; 15(5): 898-910), which uses another polymerase-III promoter (U6).

The double-stranded siRNAs of the invention may be introduced into mammalian cells *in vitro* or *in vivo* using known techniques, as described below, to suppress expression of IL-11 or IL-11R.

Similarly, transcription vectors containing the DNAs of the invention may be introduced into tumour cells *in vitro* or *in vivo* using known techniques, as described below, for transient or stable expression of RNA, again to suppress expression of IL-11 or IL-11R.

Accordingly, the invention also provides a method of suppressing IL-11 or IL-11R expression in a mammalian, e.g. human, cell, the method comprising administering to the cell a double-stranded siRNA of the invention or a transcription vector of the invention.

Similarly, the invention further provides a method of treating fibrosis, the method comprising administering to a subject a double-stranded siRNA of the invention or a transcription vector of the invention.

The invention further provides the double-stranded siRNAs of the invention and the transcription vectors of the invention, for use in a method of treatment, preferably a method of treating fibrosis.

The invention further provides the use of the double-stranded siRNAs of the invention and the transcription vectors of the invention in the preparation of a medicament for the treatment of fibrosis.

The invention further provides a composition comprising a double-stranded siRNA of the invention or a transcription vector of the invention in admixture with one or more pharmaceutically acceptable carriers. Suitable carriers include lipophilic carriers or vesicles, which may assist in penetration of the cell membrane.

Materials and methods suitable for the administration of siRNA duplexes and DNA vectors of the invention are well known in the art and improved methods are under development, given the potential of RNAi technology.

Generally, many techniques are available for introducing nucleic acids into mammalian cells. The choice of technique will depend on whether the nucleic acid is transferred into cultured cells *in vitro* or *in vivo* in the cells of a patient. Techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, DEAE dextran and calcium phosphate precipitation. *In vivo* gene transfer techniques include transfection with viral (typically

retroviral) vectors and viral coat protein-liposome mediated transfection (Dzau et al. (2003) Trends in Biotechnology 11, 205-210).

In particular, suitable techniques for cellular administration of the nucleic acids of the invention both *in vitro* and *in vivo* are disclosed in the following articles:

General reviews: Borkhardt, A. 2002. Blocking oncogenes in malignant cells by RNA interference--new hope for a highly specific cancer treatment? Cancer Cell. 2:167-8. Hannon, G.J. 2002. RNA interference. Nature. 418:244-51. McManus, M.T., and P.A. Sharp. 2002. Gene silencing in mammals by small interfering RNAs. Nat Rev Genet. 3:737-47. Scherr, M., M.A. Morgan, and M. Eder. 2003b. Gene silencing mediated by small interfering RNAs in mammalian cells. Curr Med Chem. 10:245-56. Shuey, D.J., D.E. McCallus, and T. Giordano. 2002. RNAi: gene-silencing in therapeutic intervention. Drug Discov Today. 7:1040-6.

Systemic delivery using liposomes: Lewis, D.L., J.E. Hagstrom, A.G. Loomis, J.A. Wolff, and H. Herweijer. 2002. Efficient delivery of siRNA for inhibition of gene expression in postnatal mice. Nat Genet. 32:107-8. Paul, C.P., P.D. Good, I. Winer, and D.R. Engelke. 2002. Effective expression of small interfering RNA in human cells. Nat Biotechnol. 20:505-8. Song, E., S.K. Lee, J. Wang, N. Ince, N. Ouyang, J. Min, J. Chen, P. Shankar, and J. Lieberman. 2003. RNA interference targeting Fas protects mice from fulminant hepatitis. Nat Med. 9:347-51. Sorensen, D.R., M. Leirdal, and M. Sioud. 2003. Gene silencing by systemic delivery of synthetic siRNAs in adult mice. J Mol Biol. 327:761-6.

Virus mediated transfer: Abbas-Terki, T., W. Blanco-Bose, N. Deglon, W. Pralong, and P. Aebischer. 2002. Lentiviral-mediated RNA interference. Hum Gene Ther. 13:2197-201. Barton, G.M., and R. Medzhitov. 2002. Retroviral delivery of small interfering RNA into primary cells. Proc Natl Acad Sci U S A. 99:14943-5. Devroe, E., and P.A. Silver. 2002. Retrovirus-delivered siRNA. BMC Biotechnol. 2:15. Lori, F., P. Guallini, L. Galluzzi, and J. Lisziewicz. 2002. Gene therapy approaches to HIV infection. Am J Pharmacogenomics. 2:245-52. Matta, H., B. Hozayev, R. Tomar, P. Chugh, and P.M. Chaudhary. 2003. Use of lentiviral vectors for delivery of small interfering RNA. Cancer Biol Ther. 2:206-10. Qin, X.F., D.S. An, I.S. Chen, and D. Baltimore. 2003. Inhibiting HIV-1 infection in human T cells by lentiviral-mediated delivery of small interfering RNA against CCR5. Proc Natl Acad Sci U S A. 100:183-8. Scherr, M., K. Battmer, A. Ganser, and M. Eder. 2003a. Modulation of gene expression by lentiviral-mediated delivery of small interfering RNA.

Cell Cycle. 2:251-7. Shen, C., A.K. Buck, X. Liu, M. Winkler, and S.N. Reske. 2003. Gene silencing by adenovirus-delivered siRNA. FEBS Lett. 539:111-4.

Peptide delivery: Morris, M.C., L. Chaloin, F. Heitz, and G. Divita. 2000. Translocating peptides and proteins and their use for gene delivery. Curr Opin Biotechnol. 11:461-6. Simeoni, F., M.C. Morris, F. Heitz, and G. Divita. 2003. Insight into the mechanism of the peptide-based gene delivery system MPG: implications for delivery of siRNA into mammalian cells. Nucleic Acids Res. 31:2717-24. Other technologies that may be suitable for delivery of siRNA to the target cells are based on nanoparticles or nanocapsules such as those described in US patent numbers 6,649,192B and 5,843,509B.

Formulations

In therapeutic applications, agents capable of inhibiting the action of IL-11 or agents capable of preventing or reducing the expression of IL-11 or IL-11R are preferably formulated as a medicament or pharmaceutical together with one or more other pharmaceutically acceptable ingredients well known to those skilled in the art, including, but not limited to, pharmaceutically acceptable carriers, adjuvants, excipients, diluents, fillers, buffers, preservatives, anti-oxidants, lubricants, stabilisers, solubilisers, surfactants (e.g., wetting agents), masking agents, colouring agents, flavouring agents, and sweetening agents.

The term "pharmaceutically acceptable" as used herein pertains to compounds, ingredients, materials, compositions, dosage forms, etc., which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of the subject in question (e.g., human) without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Each carrier, adjuvant, excipient, etc. must also be "acceptable" in the sense of being compatible with the other ingredients of the formulation.

Suitable carriers, adjuvants, excipients, etc. can be found in standard pharmaceutical texts, for example, Remington's Pharmaceutical Sciences, 18th edition, Mack Publishing Company, Easton, Pa., 1990; and Handbook of Pharmaceutical Excipients, 2nd edition, 1994.

The formulations may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active compound with a carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active compound with carriers (e.g., liquid carriers, finely divided solid carrier, etc.), and then shaping the product, if necessary.

The formulations may be prepared for topical, parenteral, systemic, intravenous, intra-arterial, intramuscular, intrathecal, intraocular, intra-conjunctival, subcutaneous, oral or transdermal routes of administration which may include injection. Injectable formulations may comprise the selected agent in a sterile or isotonic medium.

Administration is preferably in a "therapeutically effective amount", this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of the disease being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 20th Edition, 2000, pub. Lippincott, Williams & Wilkins.

Fibrosis

As used herein, "fibrosis" refers to the formation of excess fibrous connective tissue as a result of the excess deposition of extracellular matrix components, for example collagen. Fibrous connective tissue is characterised by having extracellular matrix (ECM) with a high collagen content. The collagen may be provided in strands or fibers, which may be arranged irregularly or aligned. The ECM of fibrous connective tissue may also include glycosaminoglycans.

As used herein, "excess fibrous connective tissue" refers to an amount of connective tissue at a given location (e.g. a given tissue or organ, or part of a given tissue or organ) which is greater than the amount of connective tissue present at that location in the absence of fibrosis, e.g. under normal, non-pathological conditions. As used herein, "excess deposition of extracellular matrix components" refers to a level of deposition of

one or more extracellular matrix components which is greater than the level of deposition in the absence of fibrosis, e.g. under normal, non-pathological conditions.

The cellular and molecular mechanisms of fibrosis are described in Wynn, J. Pathol. (2008) 214(2): 199-210, and Wynn and Ramalingam, Nature Medicine (2012) 18:1028-1040, which are hereby incorporated by reference in their entirety.

The main cellular effectors of fibrosis are myofibroblasts, which produce a collagen-rich extracellular matrix.

In response to tissue injury, damaged cells and leukocytes produce pro-fibrotic factors such as TGFβ, IL-13 and PDGF, which activate fibroblasts to αSMA-expressing myofibroblasts, and recruit myofibroblasts to the site of injury. Myofibroblasts produce a large amount of extracellular matrix, and are important mediators in aiding contracture and closure of the wound. However, under conditions of persistent infection or during chronic inflammation there can be overactivation and recruitment of myofibroblasts, and thus over-production of extracellular matrix components, resulting in the formation of excess fibrous connective tissue.

In some embodiments fibrosis may be triggered by pathological conditions, e.g. conditions, infections or disease states that lead to production of pro-fibrotic factors such as TGFβ1. In some embodiments, fibrosis may be caused by physical injury/stimuli, chemical injury/stimuli or environmental injury/stimuli. Physical injury/stimuli may occur during surgery, e.g. iatrogenic causes. Chemical injury/stimuli may include drug induced fibrosis, e.g. following chronic administration of drugs such as bleomycin, cyclophosphamide, amiodarone, procainamide, penicillamine, gold and nitrofurantoin (Daba et al., Saudi Med J 2004 Jun; 25(6): 700-6). Environmental injury/stimuli may include exposure to asbestos fibres or silica.

Fibrosis can occur in many tissues of the body. For example, fibrosis can occur in the liver (e.g. cirrhosis), lungs, kidney, heart, blood vessels, eye, skin, pancreas, intestine, brain, and bone marrow. Fibrosis may also occur in multiple organs at once.

In embodiments herein, fibrosis may involve an organ of the gastrointestinal system, e.g. of the liver, small intestine, large intestine, or pancreas. In some embodiments, fibrosis may involve an organ of the respiratory system, e.g. the lungs. In embodiments, fibrosis

may involve an organ of the cardiovascular system, e.g. of the heart or blood vessels. In some embodiments, fibrosis may involve the skin. In some embodiments, fibrosis may involve an organ of the nervous system, e.g. the brain. In some embodiments, fibrosis may involve an organ of the urinary system, e.g. the kidneys. In some embodiments, fibrosis may involve an organ of the musculoskeletal system, e.g. muscle tissue.

In some preferred embodiments, the fibrosis is cardiac or myocardial fibrosis, hepatic fibrosis, or renal fibrosis. In some embodiments cardiac or myocardial fibrosis is associated with dysfunction of the musculature or electrical properties of the heart, or thickening of the walls of valves of the heart. In some embodiments fibrosis is of the atrium and/or ventricles of the heart. Treatment or prevention of atrial or ventricular fibrosis may help reduce risk or onset of atrial fibrillation, ventricular fibrillation, or myocardial infarction.

In some preferred embodiments hepatic fibrosis is associated with chronic liver disease or liver cirrhosis. In some preferred embodiments renal fibrosis is associated with chronic kidney disease.

Diseases/conditions characterised by fibrosis in accordance with the present invention include but are not limited to: respiratory conditions such as pulmonary fibrosis, cystic fibrosis, idiopathic pulmonary fibrosis, progressive massive fibrosis, scleroderma, obliterative bronchiolitis, Hermansky-Pudlak syndrome, asbestosis, silicosis, chronic pulmonary hypertension, AIDS associated pulmonary hypertension, sarcoidosis, tumor stroma in lung disease, and asthma; chronic liver disease, primary biliary cirrhosis (PBC), schistosomal liver disease, liver cirrhosis; cardiovascular conditions such as hypertrophic cardiomyopathy, dilated cardiomyopathy (DCM), fibrosis of the atrium, atrial fibrillation, fibrosis of the ventricle, ventricular fibrillation, myocardial fibrosis, Brugada syndrome, myocarditis, endomyocardial fibrosis, myocardial infarction, fibrotic vascular disease, hypertensive heart disease, arrhythmogenic right ventricular cardiomyopathy (ARVC), tubulointerstitial and glomerular fibrosis, atherosclerosis, varicose veins, cerebral infarcts; neurological conditions such as gliosis and Alzheimer's disease; muscular dystrophy such as Duchenne muscular dystrophy (DMD) or Becker's muscular dystrophy (BMD); gastrointestinal conditions such as Chron's disease, microscopic colitis and primary sclerosing cholangitis (PSC); skin conditions such as scleroderma, nephrogenic systemic fibrosis and cutis keloid; arthrofibrosis; Dupuytren's contracture; mediastinal fibrosis; retroperitoneal fibrosis; myelofibrosis; Peyronie's disease; adhesive capsulitis; kidney

disease (e.g., renal fibrosis, nephritic syndrome, Alport's syndrome, HIV associated nephropathy, polycystic kidney disease, Fabry's disease, diabetic nephropathy, chronic glomerulonephritis, nephritis associated with systemic lupus); progressive systemic sclerosis (PSS); chronic graft versus host disease; diseases of the eye such as Grave's opthalmopathy, epiretinal fibrosis, retinal fibrosis, subretinal fibrosis (e.g. associated with macular degeneration (e.g. wet age-related macular degeneration (AMD)), diabetic retinopathy, glaucoma, corneal fibrosis, post-surgical fibrosis (e.g. of the posterior capsule following cataract surgery, or of the bleb following trabeculectomy for glaucoma), conjunctival fibrosis, subconjunctival fibrosis; arthritis; fibrotic pre-neoplastic and fibrotic neoplastic disease; and fibrosis induced by chemical or environmental insult (e.g., cancer chemotherapy, pesticides, radiation/cancer radiotherapy).

It will be appreciated that the many of the diseases/conditions listed above are interrelated. For example, fibrosis of the ventricle may occur post myocardial infarction, and is associated with DCM, HCM and myocarditis.

In particular embodiments, the disease/disorder may be one of pulmonary fibrosis, atrial fibrillation, ventricular fibrillation, hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), non-alcoholic steatohepatitis (NASH), cirrhosis, chronic kidney disease, scleroderma, systemic sclerosis, keloid, cystic fibrosis, Chron's disease, post-surgical fibrosis or retinal fibrosis.

Treatment, prevention or alleviation of fibrosis according to the present invention may be of fibrosis that is associated with an upregulation of IL-11, e.g. an upregulation of IL-11 in cells or tissue in which the fibrosis occurs or may occur, or upregulation of extracellular IL-11 or IL-11R.

Treatment or alleviation of fibrosis may be effective to prevent progression of the fibrosis, e.g. to prevent worsening of the condition or to slow the rate of development of the fibrosis. In some embodiments treatment or alleviation may lead to an improvement in the fibrosis, e.g. a reduction in the amount of deposited collagen fibres.

Prevention of fibrosis may refer to prevention of a worsening of the condition or prevention of the development of fibrosis, e.g. preventing an early stage fibrosis developing to a later, chronic, stage.

Subject

The subject to be treated may be any animal or human. The subject is preferably mammalian, more preferably human. The subject may be a non-human mammal, but is more preferably human. The subject may be male or female. The subject may be a patient.

Sample

A sample obtained from a subject may be of any kind. A biological sample may be taken from any tissue or bodily fluid, e.g. a blood sample, blood-derived sample, serum sample, lymph sample, semen sample, saliva sample, synovial fluid sample. A blood-derived sample may be a selected fraction of a patient's blood, e.g. a selected cell-containing fraction or a plasma or serum fraction. A sample may comprise a tissue sample or biopsy; or cells isolated from a subject. Samples may be collected by known techniques, such as biopsy or needle aspirate. Samples may be stored and/or processed for subsequent determination of IL-11 expression levels.

Samples may be used to determine the upregulation of IL-11 or IL-11R in the subject from which the sample was taken.

In some preferred embodiments a sample may be a tissue sample, e.g. biopsy, taken from heart, liver or kidney tissue. In some embodiments a sample may be a tissue sample, e.g. biopsy, taken from the eye.

A sample may contain cells, and may preferably contain fibroblasts and/or myofibroblasts. In some embodiments, fibroblasts or myofibroblasts may be obtained from heart, liver or kidney tissue, e.g. they may be cardiac fibroblasts or cardiac myofibroblasts (e.g. *see* Colby et al., Circulation Research 2009;105:1164-1176), hepatic fibroblasts or hepatic myofibroblasts (e.g. *see* Zeisberg et al., The Journal of Biological Chemistry, August 10, 2007, 282, 23337-23347; Brenner., Fibrogenesis & Tissue Repair 2012, 5(Suppl 1):S17) or renal fibroblasts or renal myofibroblasts (e.g. *see* Strutz and Zeisberg. JASN November 2006 vol. 17 no. 11 2992-2998). In some embodiments, fibroblasts or myofibroblasts may be obtained from eye tissue, e.g. they may be corneal fibroblasts.

Upregulation of IL-11 or IL-11R expression

Some aspects and embodiments of the present invention concern detection of expression of IL-11 or IL-11R, e.g. in a sample obtained from a subject.

In some aspects and embodiments the present invention concerns the upregulation of expression (over-expression) of IL-11 or IL-11R (as a protein or oligonucleotide encoding the respective IL-11 or IL-11R) and detection of such upregulation as an indicator of suitability for treatment with an agent capable of inhibiting the action of IL-11 or with an agent capable of preventing or reducing the expression of IL-11 or IL-11R.

Upregulation of IL-11 or IL-11R expression comprises expression of IL-11 or IL-11R at a level that is greater than would normally be expected for a cell or tissue of a given type. Upregulation may be determined by determining the level of expression of IL-11 or IL-11R in a cell or tissue. A comparison may be made between the level of IL-11 or IL-11R expression in a cell or tissue sample from a subject and a reference level of IL-11 or IL-11R, e.g. a value or range of values representing a normal level of expression of IL-11 or IL-11R for the same or corresponding cell or tissue type. In some embodiments reference levels may be determined by detecting IL-11 or IL-11R expression in a control sample, e.g. in corresponding cells or tissue from a healthy subject or from healthy tissue of the same subject. In some embodiments reference levels may be obtained from a standard curve or data set.

Levels of expression may be quantitated for absolute comparison, or relative comparisons may be made.

In some embodiments upregulation of IL-11 or IL-11R may be considered to be present when the level of expression in the test sample is at least 1.1 times that of a reference level. More preferably, the level of expression may be selected from one of at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2.0, at least 2.1, at least 2.2, at least 2.3, at least 2.4 at least 2.5, at least 2.6, at least 2.7, at least 2.8, at least 2.9, at least 3.0, at least 3.5, at least 4.0, at least 5.0, at least 6.0, at least 7.0, at least 8.0, at least 9.0, or at least 10.0 times that of the reference level.

IL-11 or IL-11R expression levels may be determined by one of a number of known *in vitro* assay techniques, such as PCR based assays, in situ hybridisation assays, flow cytometry assays, immunological or immunohistochemical assays.

By way of example suitable techniques involve a method of detecting the level of IL-11 or IL-11R in a sample by contacting the sample with an agent capable of binding IL-11 or IL-11R and detecting the formation of a complex of the agent and IL-11 or IL-11R. The agent may be any suitable binding molecule, e.g. an antibody, polypeptide, peptide, oligonucleotide, aptamer or small molecule, and may optionally be labelled to permit detection, e.g. visualisation, of the complexes formed. Suitable labels and means for their detection are well known to those in the art and include fluorescent labels (e.g. fluorescein, rhodamine, eosine and NDB, green fluorescent protein (GFP), chelates of rare earths such as europium (Eu), terbium (Tb) and samarium (Sm), tetramethyl rhodamine, Texas Red, 4-methyl umbelliferone, 7-amino-4-methyl coumarin, Cy3, Cy5), isotope markers, radioisotopes (e.g. ^{32}P, ^{33}P, ^{35}S), chemiluminescence labels (e.g. acridinium ester, luminol, isoluminol), enzymes (e.g. peroxidase, alkaline phosphatase, glucose oxidase, beta-galactosidase, luciferase), antibodies, ligands and receptors. Detection techniques are well known to those of skill in the art and can be selected to correspond with the labelling agent. Suitable techniques include PCR amplification of oligonucleotide tags, mass spectrometry, detection of fluorescence or colour, e.g. upon enzymatic conversion of a substrate by a reporter protein, or detection of radioactivity.

Assays may be configured to quantify the amount of IL-11 or IL-11R in a sample. Quantified amounts of IL-11 or IL-11R from a test sample may be compared with reference values, and the comparison used to determine whether the test sample contains an amount of IL-11 or IL-11R that is higher or lower than that of the reference value to a selected degree of statistical significance.

Quantification of detected IL-11 or IL-11R may be used to determine up- or down-regulation or amplification of genes encoding IL-11 or IL-11R. In cases where the test sample contains fibrotic cells, such up-regulation, down-regulation or amplification may be compared to a reference value to determine whether any statistically significant difference is present.

Subject selection

A subject may be selected for treatment based on a determination that the subject has an upregulated level of IL-11 or IL-11R expression. IL-11 or IL-11R may therefore act as a marker of a fibrosis that is suitable for treatment with an agent capable of inhibiting the action of IL-11 or with an agent capable of preventing or reducing the expression of IL-11 or IL-11R.

Upregulation may be in a given tissue or in selected cells from a given tissue. A preferred tissue may be one of heart, liver or kidney. A preferred tissue may be eye. A preferred cell type may be fibroblasts or myofibroblasts. Upregulation may also be determined in a circulating fluid, e.g. blood, or in a blood derived sample. Upregulation may be of extracellular IL-11 or IL-11R.

Determination of IL-11 or IL-11R levels may be performed by assay, preferably *in vitro*, on a sample obtained from a subject, as described herein.

Following selection, a subject may be provided with treatment for fibrosis by administration of an agent capable of inhibiting the action of IL-11 or an agent capable of preventing or reducing the expression of IL-11 or IL-11R.

In some embodiments a subject may have been diagnosed with fibrosis, be suspected of having fibrosis or be considered at risk of developing fibrosis and it is of interest whether the subject will benefit from treatment with an agent capable of inhibiting the action of IL-11 or with an agent capable of preventing or reducing the expression of IL-11 or IL-11R. In such embodiments, the suitability of the subject for such treatment may be determined by determining whether IL-11 or IL-11R expression is upregulated in the subject. In some embodiments, IL-11 or IL-11R expression is locally or systemically upregulated in the subject.

Diagnosis and Prognosis

The detection of upregulation of IL-11 or IL-11R expression may also be used in a method of diagnosing fibrosis or the risk of developing fibrosis in a subject, and in methods of prognosing or predicting a subject's response to treatment with an agent capable of inhibiting the action of IL-11 or an agent capable of preventing or reducing the expression of IL-11 or IL-11R.

In some embodiments a subject may be suspected of having fibrosis, e.g. based on the presence of other symptoms indicative of fibrosis in the subject's body or in selected cells/tissues of the subject's body, or be considered at risk of developing fibrosis, e.g. because of genetic predisposition or exposure to environmental conditions, such as asbestos fibres.

Determination of upregulation of IL-11 or IL-11R may confirm a diagnosis or suspected diagnosis of fibrosis or may confirm that the subject is at risk of developing fibrosis. The determination may also diagnose the condition or predisposition as one suitable for treatment with an agent capable of inhibiting the action of IL-11 or an agent capable of preventing or reducing the expression of IL-11 or IL-11R.

As such, a method of providing a prognosis for a subject having, or suspected of having fibrosis may be provided, the method comprising determining whether IL-11 or IL-11R is upregulated in a sample obtained from the subject and, based on the determination, providing a prognosis for treatment of the subject with an agent capable of inhibiting the action of IL-11 or an agent capable of preventing or reducing the expression of IL-11 or IL-11R.

In some aspects methods of diagnosis or methods of prognosing or predicting a subject's response to treatment with an agent capable of inhibiting the action of IL-11 or an agent capable of preventing or reducing the expression of IL-11 or IL-11R may not require determination of IL-11 or IL-11R levels, but may be based on determining genetic factors in the subject that are predictive of upregulation of IL-11 or IL-11R expression, or upregulation of IL-11 or IL-11R activity. Such genetic factors may include the determination of genetic mutations, single nucleotide polymorphisms (SNPs) or gene amplification in IL-11 and/or IL-11R that are correlated with and/or predictive of upregulation of IL-11 or IL-11R expression or activity or IL-11 mediated signaling activity. The use of genetic factors to predict predisposition to a disease state or response to treatment is known in the art, e.g. see Peter Stärkel *Gut* 2008;57:440-442; Wright et al., Mol. Cell. Biol. March 2010 vol. 30 no. 6 1411-1420.

Genetic factors may be assayed by methods known to those of ordinary skill in the art, including PCR based assays, e.g. quantitative PCR, competitive PCR. By determining the presence of genetic factors, e.g. in a sample obtained from a subject, a diagnosis of fibrosis may be confirmed, and/or a subject may be classified as being at risk of developing fibrosis, and/or a subject may be identified as being suitable for treatment with an agent capable of inhibiting the action of IL-11 or an agent capable of preventing or reducing the expression of IL-11 or IL-11R.

Some methods may comprise determination of the presence of one or more SNPs linked to secretion of IL-11 or susceptibility to development of fibrosis. SNPs are usually bi-allelic and therefore can be readily determined using one of a number of conventional assays known to those of skill in the art (e.g. see Anthony J. Brookes. The essence of SNPs. Gene Volume 234, Issue 2, 8 July 1999, 177-186; Fan et al., Highly Parallel SNP Genotyping. Cold Spring Harb Symp Quant Biol 2003. 68: 69-78; Matsuzaki et al., Parallel Genotyping of Over 10,000 SNPs using a one-primer assay on a high-density oligonucleotide array. Genome Res. 2004. 14: 414-425).

The methods may comprise determining which SNP allele is present in a sample obtained from a subject. In some embodiments determining the presence of the minor allele may be associated with increased IL-11 secretion or susceptibility to development of fibrosis.

Accordingly, in one aspect of the present invention a method for screening a subject is provided, the method comprising:

obtaining a nucleic acid sample from the subject;

determining which allele is present in the sample at the polymorphic nucleotide position of one or more of the SNPs listed in Figure 33, and/or Figure 34 and/or Figure 35 or an SNP in linkage disequilibrium with one of the listed SNPs with an $r^2 \geq 0.8$.

The determining step may comprise determining whether the minor allele is present in the sample at the selected polymorphic nucleotide position. It may comprise determining whether 0, 1 or 2 minor alleles are present.

The screening method may be, or form part of, a method for determining susceptibility of the subject to development of fibrosis, or a method of diagnosis or prognosis as described herein.

The method may further comprise the step of identifying the subject as having susceptibility to, or an increased risk of, developing fibrosis, e.g. if the subject is determined to have a minor allele at the polymorphic nucleotide position. The method may further comprise the step of selecting the subject for treatment with an agent capable of inhibiting the action of Interleukin 11 (IL-11) and/or administering an agent capable of inhibiting the action of Interleukin 11 (IL-11) to the subject in order to provide a treatment

for fibrosis in the subject or to prevent development or progression of fibrosis in the subject.

SNPs that may be determined include one or more of the SNPs listed in Figure 33, Figure 34, or Figure 35. In some embodiments the method may comprise determining one or more of the SNPs listed in Figure 33. In some embodiments the method may comprise determining one or more of the SNPs listed in Figure 34. In some embodiments the method may comprise determining one or more of the SNPs listed in Figure 35. SNPs may be selected for determination as having a low P value or FDR (false discovery rate).

In some embodiments SNPs are selected as being good predictors of response to anti-IL-11 treatment based on regulation of VSTstim in *trans* (Figures 33). In some embodiments a method may comprise determining which allele is present for one or more of the following SNPs: rs10831850, rs4756936, rs6485827, rs7120273, and rs895468. In some embodiments SNPs are selected as being good predictors of response to anti-IL-11 treatment based on regulation VSTstim-VSTunstim in *cis* (Figure 34).

In some embodiments SNPs are selected as being good predictors of response to anti-IL-11 treatment based on regulation VSTstim-VSTunstim in *trans* (Figure 35). In some embodiments a method may comprise determining which allele is present for one or more of the following SNPs: rs7120273, rs10831850, rs4756936, rs6485827 (Figure 35).

SNPs: rs7120273, rs10831850, rs4756936, rs6485827 are in high linkage disequilibrium (LD) with one another on chromosome 11 (in a so-called LD block), and are therefore very commonly co-inherited.

The square of the correlation of gene frequencies (r^2) reflects the degree of linkage disequilibrium (LD) between two SNPs. As a result of LD between SNPs in local and therefore co-inherited regions of the genome, the genotype of a given SNP can be inferred by determining the genotype of a tagging/proxy SNP. The threshold of LD used in the art to identify pairwise tagging/proxy SNPs is an r^2 value of 0.8 (Wang et al. 2005, Nat. Rev. Genet. 6(2): 109-18; Barrett et al. 2006, Nat Genet., 38 (6): 659-662). The genotype of a given SNP can therefore be inferred by determining the genotype of a tagging/proxy SNP in linkage disequilibrium with an r^2 value ≥ 0.8.

The nucleotide sequence of SNPs is indicated using the "rs" number. The full sequence is available from the National Center for biotechnology Information (NCBI) database of single nucleotide polymorphisms (dbSNP) accessible at: https://www.ncbi.nlm.nih.gov/snp.

Methods of diagnosis or prognosis may be performed *in vitro* on a sample obtained from a subject, or following processing of a sample obtained from a subject. Once the sample is collected, the patient is not required to be present for the *in vitro* method of diagnosis or prognosis to be performed and therefore the method may be one which is not practised on the human or animal body.

Other diagnostic or prognostic tests may be used in conjunction with those described here to enhance the accuracy of the diagnosis or prognosis or to confirm a result obtained by using the tests described here.

Methods according to the present invention may be performed, or products may be present, *in vitro*, *ex vivo*, or *in vivo*. The term "*in vitro*" is intended to encompass experiments with materials, biological substances, cells and/or tissues in laboratory conditions or in culture whereas the term "*in vivo*" is intended to encompass experiments and procedures with intact multi-cellular organisms. "*Ex vivo*" refers to something present or taking place outside an organism, e.g. outside the human or animal body, which may be on tissue (e.g. whole organs) or cells taken from the organism.

The invention includes the combination of the aspects and preferred features described except where such a combination is clearly impermissible or expressly avoided.

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Aspects and embodiments of the present invention will now be illustrated, by way of example, with reference to the accompanying figures. Further aspects and embodiments will be apparent to those skilled in the art. All documents mentioned in this text are incorporated herein by reference.

Throughout this specification, including the claims which follow, unless the context requires otherwise, the word "comprise," and variations such as "comprises" and

"comprising," will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Ranges may be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by the use of the antecedent "about," it will be understood that the particular value forms another embodiment.

Brief Description of the Figures

Embodiments and experiments illustrating the principles of the invention will now be discussed with reference to the accompanying figures in which:

Figure 1. TGFβ1 stimulation upregulates IL-11 in fibroblasts. Primary fibroblasts were derived from human atrial tissue of 80 individuals and incubated for 24h with and without TGFβ1 (5ng/ml). (a) Chart showing IL-11 was the most upregulated gene in TGFβ1 stimulated fibroblasts compared to 11,433 expressed genes (FPKM ≥ 0.5). (b) Chart showing IL-11 expression significantly increased more than 8-fold on average after fibroblast activation with TGFβ1 (FDR = $9.1x10^{-125}$). (c) Chart showing RT-qPCR confirmed IL-11 RNA expression-based fold changes (TGFB1+ / TGFB1-; R^2=0.94) and (d) Chart showing ELISA detected a significant increase in IL-11 protein secreted by stimulated fibroblasts.

Figure 2. Human atrial fibroblasts were incubated either with 5 ng/ml TGFβ1 or 5 ng/ml IL-11 for 24 hours. Charts show cell staining for (a) α-SMA (myofibroblasts), (b) EdU (proliferation), (c) collagen and (d) periostin to identify myofibroblasts and highly proliferative cells and to quantify the production of extracellular matrix proteins. IL-11 was found to increase the myofibroblast ratio and induce the production of collagen and periostin at a similar rate as TGFβ1 signaling. This experiment was repeated a number of times with similar results.

Figure 3. Inhibition of IL-11 with a neutralizing antibody prevents TGFβ1-induced fibrosis. Human atrial fibroblasts were stimulated with TGFβ1 (5ng/ml), TGFβ1 and an antibody against IL-11 or TGFβ1 and an isotype control. Charts and photographs show cell stained after 24 hours for (a) α-SMA, (b) EdU, (c) collagen and (d) periostin to identify myofibroblasts and highly proliferative cells and to quantify the production of extracellular matrix proteins. Fluorescence was quantified on the Operetta platform for up to 21 fields per condition. This experiment was repeated with fibroblasts derived from different individuals with similar results. In the presence of an antibody blocking IL-11, TGFβ1-stimulated fibroblasts have a decreased ratio of myofibroblasts, are less proliferative and express less collagen and periostin compared to control cells. This shows that IL-11 is an essential component of TGFβ1 signaling pathway acting in an autocrine and/or paracrine feed forward fashion and its inhibition reduces the pro-fibrotic effects of this key regulator of fibrosis in humans.

Figure 4. TGFβ1 stimulation upregulates IL-11 in fibroblasts. Primary fibroblasts were derived from human atrial tissue of 80 individuals and incubated for 24h with and without TGFβ1 (5ng/ml). (a) Chart showing IL-11 was the most upregulated RNA transcript in TGFβ1 stimulated fibroblasts compared to 11,433 expressed genes (FPKM ≥ 0.5) across the genome as assessed by global transcriptome profiling. (b) Chart showing IL-11 expression in non-stimulated (TGF-β -) and stimulated (TGF-β +) primary human fibroblasts compared to all human tissues as assessed by the GTEX project (Consortium, Gte. Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science (New York, N.Y.)* **348,** (2015)) reveals high specificity of elevated IL-11 levels to fibroblasts and specifically activated fibroblasts, the signature of which is not appreciated at the level of the whole organ that contains multiple cell types and few, IL11-expressing, fibroblasts.

Figure 5. IL-11 acts as an autocrine factor on fibroblasts and induces its own expression via translational regulation alone. Primary fibroblasts were stimulated with TGF-β for 24 hours. (a) Chart showing IL-11 RNA expression increased significantly (FDR = 9.1×10^{-125}) more than 8-fold on average across 80 individuals. (b) Chart showing results of an ELISA assay confirming a significant increase in IL-11 protein secreted by stimulated fibroblasts (t-test). (c) Chart showing incubation of primary fibroblasts with IL-11 does not increase IL-11 RNA levels (RT-qPCR). (d) Chart showing incubation of primary fibroblasts with IL-11 induces IL-11 protein secretion significantly (Dunnett) as detected by ELISA. Adjusted P-values are given as **** $P < 0.0001$.

Figure 6. IL-11 drives proliferation and activation of fibroblasts as well as extracellular matrix production and is required for the TGFβ1-mediated fibrotic response. Cardiac fibroblasts derived from 3 individuals were incubated for 24h with TGFβ1 (5 ng/ml), IL-11 (5 ng/ml) or TGFβ1 and a neutralizing IL-11/control antibody. Charts and photographs show results of cell staining following incubation for (a) α-SMA content to estimate the fraction of myofibroblasts, (b) EdU to track actively proliferating cells (c) Periostin to estimate ECM production. Fluorescence was measured with the Operetta platform for 14 fields across 2 wells for each patient. Charts also show the secretion of fibrosis markers IL-6 (d), TIMP1 (e) and MMP2 (f) as assessed via ELISA. Fluorescence was normalized to the control group without stimulation and the mean with standard deviation is plotted. IL-11 induces a fibrotic response at similar levels as TGFβ1 and inhibition of IL-11 rescues the TGFβ1 phenotype on the protein level. Adjusted P-values are given as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ or **** $P < 0.0001$ of experimental groups compared to unstimulated cells (Dunnett). Outliers were removed (ROUT, Q = 2%).

Figure 7. IL-11 promotes collagen protein synthesis and stalls the pro-fibrotic effect of TGFβ1 at the RNA level. Cardiac fibroblasts derived from 3 individuals were incubated for 24h with TGFβ1 (5 ng/ml), IL-11 (5 ng/ml) or TGFβ1 and a neutralizing IL-11 antibody. Following incubation (a) Chart showing results following incubation of cell staining for collagen using the Operetta assay; florescence was quantified as described above for Figure 6, (b) Chart showing secreted collagen levels assessed with a Sirius Red staining and (c) Chart showing collagen RNA levels measured by RT-qPCR. IL-11 induces a fibrotic response at similar levels as TGFβ1 only at the protein level. Higher expression of Collagen RNA transcripts by TGFβ1 did not lead to increased protein production if IL-11 was neutralized with an antibody. Adjusted P-values are given as * $P < 0.05$, *** $P < 0.001$ or **** $P < 0.0001$ of experimental groups compared to unstimulated cell control group (Dunnett).

Figure 8. IL-11 is a fibrosis marker and activator across multiple tissues. Expression of IL-11 can be induced by a diverse set of upstream pro-fibrotic stimulants in addition to TGFβ1. (a) Chart showing effect of TGFβ1 on IL-11 expression. (b) Chart showing ET-1 (Endothelin) upregulates IL-11 in hepatic and pulmonary fibroblasts; (c) Chart showing PDGF (platelet derived growth factor) induces IL-11 expression in renal fibroblasts. IL-11

RNA levels were measured by RT-qPCR; adjusted P-values are given as * P < 0.05, ** P < 0.01 or **** P < 0.0001 (Dunnett). To investigate the systemic effect of IL-11, saline only (grey) or recombinant IL-11 (black) was injected 6 times a week in C57BL/6 mice (200µg/kg). Collagen content in tissue was assessed with a hydroxyproline assay (QuickZyme) on the protein level and the results are shown in chart (d). Tissues of animals treated with rIL-11 have higher collagen protein content than controls (ANOVA; p= 0.012). (e) Photographs of western blot showing αSMA levels are increased in the kidney and heart of IL-11 treated mice, indicating the presence of myofibroblasts.

Figure 9. Diagram illustrating role of IL-11 as an essential regulator of the fibrotic response. IL-11 is an essential regulator required for the fibrotic response. In response to tissue damage or chronic inflammation, cytokines such as TGFβ1, ET-1 or PDGF are released to upregulate the transcription of fibrosis marker genes. The autocrine agent IL-11 is then produced in response to these upstream stimuli to ensure efficient translation of upregulated transcripts into functionally relevant proteins in a cell-specific manner. Inhibition of IL-11 blocks the synthesis of key extracellular matrix and myofibroblast proteins and prevents the pro-fibrotic action of a diverse set of upstream stimuli.

Figure 10. Inhibition of IL-11 stops collagen protein synthesis in response to pro-fibrotic cytokines ANG2 (Angiotensin II), PDGF and ET-1. Cardiac fibroblasts were incubated for 24h with ANG2, PDGF or ET-1 and a neutralizing IL-11 antibody. Following incubation cells were stained for collagen and florescence was quantified. These stimuli induce a fibrotic response at similar levels to TGFβ1. However, collagen expression is not increased if IL-11 is neutralized with an antibody. P-values are given as: **** P < 0.0001 (t-test).

Figure 11. Nucleotide sequence of human IL-11, taken from Genbank accession number gi|391353405|ref|NM_000641.3 (Homo sapiens interleukin 11 (IL11), transcript variant 1, Mrna) [SEQ ID NO:1]. Underlined sequence encodes IL-11 mRNA. Shaded sequences were used for design of IL-11 knockdown siRNA and are shown separately as SEQ ID NOs 2 to 5. SEQ ID NOs 3 and 4 overlap with each other within SEQ ID NO:1.

Figure 12. Nucleotide sequence of human IL-11Rα, taken from Genbank accession number gi|975336|gb|U32324.1|HSU32324 (Human interleukin-11 receptor alpha chain mRNA, complete cds) [SEQ ID NO:6]. Underlined sequence encodes IL-

11Rα mRNA. Shaded sequences were used for design of IL-11Rα knockdown siRNA and are shown separately as SEQ ID NOs 7 to 10.

Figure 13. Table showing siRNA sequences [SEQ ID NOs 11 to 14] for knockdown of IL-11.

Figure 14. Table showing siRNA sequences [SEQ ID NOs 15 to 18] for knockdown of IL-11Rα.

Figure 15. Chart showing siRNA knockdown of IL-11Rα in HEK cells.

Figure 16. Graph showing read depth for whole transcriptome sequencing of human atrial fibroblasts from 160 individuals with and without stimulation with TGFβ1.

Figure 17. Graphs showing expression of endothelial, cardiomyocyte and fibroblast marker genes as determined by RNA-seq of the tissue of origin (human atrial tissues samples, n=8) and primary, unstimulated fibroblast cultures. (**A**) PECAM1, (**B**) MYH6 (**C**) TNNT2, (**D**) COL1A2, and (**E**) ACTA2.

Figure 18. Graphs showing upregulation of IL-11 expression in fibroblasts in response to stimulation with TGFβ1. (**A** and **B**) Graphs showing fold change in gene expression in fibrosis; IL-11 is the most upregulated gene in response to TGFβ1 treatment. (**C**) IL-11 secretion by fibroblasts in response to stimulation with TGFβ1. (**D**) Comparison of IL-11 gene expression in tissues of healthy individuals and in atrial fibroblasts, with or without TGFβ1 stimulation. (**E**) Correspondence of fold change in IL-11 expression as determined by RNA-seq vs. qPCR.

Figure 19. Graphs showing induction of IL-11 secretion in primary fibroblasts by various profibrotic cytokines, as determined by ELISA. (**A**) TGFβ1, ET-1, AngII, PDGF, OSM and IL-13 induce IL-11 secretion, and IL-11 also induces IL-11 expression in a positive feedback loop. (**B**) Graph showing that the ELISA only detects native IL-11 secreted from cells, and does not detect recombinant IL-11 used for the IL-11 stimulation condition. (**C**) and (**D**) Cells were stimulated with recombinant IL-11, IL-11 RNA was measured and the native IL-11 protein level was measured in the cell culture supernatant by ELISA at the indicated time points.

Figure 20. Graphs and images showing myofibroblast generation from, and production of ECM and cytokine expression by, atrial fibroblasts in response to stimulation with TGFβ1 or IL-11. (**A**) myofibroblast generation and ECM production by primary atrial fibroblasts following stimulation with TGFβ1 or IL-11, as measured by fluorescence microscopy following staining for a α-SMA, collagen or periostin. (**B**) Collagen content of cell culture supernatant as determined by Sirius Red staining. Secretion of the fibrosis markers (**C**) IL-6, (**D**) TIMP1 and (**E**) MMP2 as measured by ELISA. (**F**) Activation of murine fibroblasts by stimulation with human or mouse recombinant IL-11. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001 [Mean ± SD, Dunnett].

Figure 21. Graphs showing the profibrotic effect of IL-11. (**A**) Mouse fibroblasts from different tissues of origin can be activated by IL-11 and display increased ECM production. [Mean ± SD, Dunnett]. Injection of mice with recombinant IL-11 or AngII results in (**B**) an increase in organ weight [Mean ± SEM], and (**C**) an increase in collagen content (as determined by HPA assay). * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001 [Mean ± SD, Dunnett].

Figure 22. Graphs and images showing that IL-11 is required the pro-fibrotic effects of TGFβ1 on fibroblasts. (**A**) myofibroblast generation and ECM production by primary atrial fibroblasts, with or without stimulation with TGFβ1, and in the presence/absence of neutralising anti-IL-11 antibody or isotype control IgG, as measured by fluorescence microscopy following staining for (**A**) α-SMA, (**B**) EdU or (**C**) Periostin. (**D** to **F**) Secretion of the fibrosis markers (**D**) IL-6, (**E**) TIMP1, and (**F**) MMP2 was analysed by ELISA. Fluorescence was normalized to the control group without stimulation. [Mean ± SD, Dunnett] * P < 0.05, ** P < 0.01, *** P < 0.001 or **** P < 0.0001.

Figure 23. Graphs and images showing the effect of neutralisation of IL-11 on collagen production triggered by TGFβ1. Collagen production by cardiac fibroblasts with or without stimulation with TGFβ1, and in the presence/absence of neutralising anti-IL-11 antibody or isotype control IgG, as determined by (**A**) Operetta assay or (**B**) Sirius Red staining. [Mean ± SD, Dunnett] * P < 0.05, ** P < 0.01, *** P < 0.001 or **** P < 0.0001.

Figure 24. Graphs showing the ability of various IL-11 and IL-11Rα antagonists to inhibit fibrosis. Human atrial fibroblasts were treated with neutralizing antibody against IL-11, neutralizing antibody against IL-11Rα, decoy IL-11 receptor molecule that binds to IL-

11, siRNA that downregulates IL-11 expression or siRNA that downregulates IL-11RA expression and the effect on the TGFβ1-driven pro-fibrotic response in fibroblasts *in vitro* was analysed. [Mean ± SD, Dunnett] * P < 0.05, ** P < 0.01, *** P < 0.001 or **** P < 0.0001.

Figure 25. Bar charts showing the response of fibroblasts from IL-11-RA knockout mice to pro-fibrotic treatment. Fibroblasts derived from IL-11RA WT (+/+), Heterozygous (+/-) and Homozygous null (-/-) mice were incubated for 24h with TGFβ1, IL-11 or AngII (5 ng/ml). (**A**) Percentage of myofibroblasts as determined by analysis αSMA content, (**B**) Percentage proliferating cells as determined by staining for EdU, (**C**) Collagen content and (**D**) ECM production as measured by detection of periostin [Mean ± SD].

Figure 26. Graphs showing the effect of IL-11 neutralisation on fibrosis in response to various pro-fibrotic stimuli. Fibroblasts were cultured *in vitro* in the presence/absence of various different pro-fibrotic factors, and in the presence/absence of neutralising anti-IL-11 antibody or pan anti-TGFβ antibody (**A**) Collagen production and (**B**) myofibroblast generation as determined by analysis of αSMA expression. [Mean ± SD, Dunnett] * P < 0.05, ** P < 0.01, *** P < 0.001 or **** P < 0.0001.

Figure 27. Bar charts showing expression of markers of fibrosis in the atrium and heart of WT and IL-11RA (-/-) animals following treatment with AngII treatment. (**A**) Collagen content, as measured by hydroxyproline assay. (**B**) Collagen (Col1A2) expression. (**C**) αSMA (ACTA2) expression. (**D**) Fibronectin (Fn1) expression.

Figure 28. Graphs showing the effect of IL-11RA knockout on folate-induced kidney fibrosis as measured by collagen content in kidney tissue.

Figure 29. Schematics of the experimental procedures for analysing fibrosis in (**A**) lung, (**B**) skin and (**C**) eye for IL-11RA -/- mice as compared to IL-11RA +/+ mice.

Figure 30. Scatterplots showing fold change in gene expression. (**A**) Fold changes in gene expression in fibroblasts following stimulation with TGFβ1, IL-11 or TGFβ1 and IL-11. (**B**) Fold changes in gene expression in fibroblasts obtained from IL-11RA+/+ and IL-11RA-/- mice following stimulation with TGFβ1.

Figure 31. Photographs showing the effect of IL-11RA knockout on wound healing and fibrosis in the eye following trabeculectomy (filtration surgery). (**A**) Eye sections of IL-11RA+/+ (WT) and IL-11RA-/- (KO) animals 7 days after filtration surgery. (**B**) Maturation of collagen fibres as evaluated by picro-sirius red/polarization light technique (Szendröi et al. 1984, Acta Morphol Hung 32, 47–55); more fibrosis is observed in WT mice than KO mice.

Figure 32. Graphs showing the effect of decoy IL-11 receptors on fibrosis in response to stimulation with TGFβ1. Fibroblasts were cultured *in vitro* in the presence/absence of TGFβ1 (5 ng/ml), in the presence or absence of (**A**) D11R1 (Decoy Receptor 50aa Linker) or (**B**) D11R2 (Decoy Receptor 33aa Linker), at various different concentrations. Myofibroblast generation after 24 hours (i.e. the percentage of activated fibroblasts) was determined by analysis of αSMA expression.

Figure 33. Table showing SNPs regulation of IL-11 VST_{stim} in *trans*.

Figure 34. Table showing SNPs regulation of IL-11 $VST_{stim} – VST_{unstim}$ in *cis*.

Figure 35. Table showing SNPs regulation of IL-11 $VST_{stim} – VST_{unstim}$ in *trans*.

Figures 36A, 36B, 36C and 36D Charts showing regulation of IL-11 response by local SNPs. The RNA of unstimulated and stimulated (TGFB1, 5ng/ml, 24h) fibroblasts derived from 69 genotyped individuals was sequenced. Samples were grouped according to genotype and the increase in IL-11 expression (VST_{stim}-VST_{unstim}) was compared between groups with 0, 1 or 2 minor alleles.

Figure 37. Charts showing regulation of IL-11 response by distant SNPs. The RNA of unstimulated and stimulated (TGFB1, 5ng/ml, 24h) fibroblasts derived from 69 genotyped individuals was sequenced. Samples were grouped according to genotype and the increase in IL11 expression (VST_{stim}-VST_{unstim}) was compared between groups with 0, or 1 minor allele.

Figures 38A, 38B, 38C and 38D. Graphs showing that IL-11 is required the pro-fibrotic effects of TGFβ1 in liver fibroblasts. Activation and proliferation of primary human liver fibroblasts, with or without stimulation with TGFβ1, and in the presence/absence of neutralising anti-IL-11 antibody or isotype control IgG, as measured by analysis of the

proportion of (**A**) α-SMA positive cells, (**B**) EdU positive cells, (**C**) Collagen positive cells and (**D**) Periostin positive cells as compared to the unstimulated cells (Baseline). [Mean ± SD, Dunnett] * P < 0.05, ** P < 0.01, *** P < 0.001 or **** P < 0.0001.

Figure 39. Bar chart showing that IL-11 is required for the pro-fibrotic effects of TGFβ1 in skin fibroblasts. Activation of mouse skin fibroblasts, with or without stimulation with TGFβ1, and in the presence/absence of neutralising anti-IL-11 antibody, as measured by analysis of the percentage of α-SMA positive cells (activated fibroblasts).

Figure 40. Bar chart showing lung fibroblast cell migration with and without IL-11 signalling. Migration of lung fibroblasts from IL-11RA+/+ (WT) and IL-11RA-/- (KO) animals was analysed in an *in vitro* scratch assay without stimulus, or in the presence of TGFβ1 or IL-11.

Examples

Example 1

The fibrotic response is characterized by widespread molecular changes in activated resident fibroblasts. To establish the role of IL-11 as a key marker of this transition we assessed and ranked global RNA expression differences in atrial fibroblasts derived from 80 individuals before and 24 hours after Transforming growth factor beta-1 (TGFβ1) activation. We cultured primary fibroblasts derived from the atrium of 80 individuals who were undergoing cardiac surgery for coronary artery disease. Fibroblasts were studied *ex vivo* at baseline and following stimulation with TGFβ1 (a powerful pro-fibrotic stimulus) using genome-wide expression profiling (RNA-Seq) combined with phenotypic assays and genotyping.

IL-11 expression was significantly induced in response to TGFβ1 treatment with RNA levels increasing as much 30x (> 8x on average). IL-11 expression was higher than expression of all other individual genes (Figures 1a,b), meaning that of the ~11,500 genes expressed in fibroblasts IL-11 is the most markedly upregulated. This upregulation IL-11 was confirmed with RT-qPCR as well as ELISA experiments (Figures 1c,d), indicating increased production and release of IL-11 protein in activated fibroblasts is the main drivers of fibrosis.

To assess whether IL-11 acts as an autocrine signaling factor that drives fibrosis, we incubated non-stimulated atrial fibroblasts with recombinant IL-11 and monitored cell proliferation, myofibroblast generation as well as collagen and periostin expression at the protein level. We observed an increase in collagen production, cell proliferation and periostin expression at levels similar to those induced by the TGFβ1 signaling pathway. IL-11 activated fibroblasts also differentiated into α-SMA+ myofibroblasts (Figure 2).

In addition to its pro-fibrotic function, IL-11 was also found to play a critical role in the TGFβ1 induced fibrotic response itself. Inhibition of IL-11 with a neutralising anti- human IL-11 monoclonal antibody (Monoclonal Mouse IgG_{2A}; Clone #22626; Catalog No. MAB218; R&D Systems, MN, USA) reduced the activation of fibroblasts through TGFβ1. Cells incubated with TGFβ1 did not generate more extracellular matrix proteins when the IL-11 antibody was present (Figure 3).

We showed that IL-11 neutralizing antibodies prevent TGFβ1-induced fibroblast activation.

Example 2

Inflammation and tissue damage stimulates a dynamic process that involves the recruitment, proliferation and activation of fibroblasts to generate extracellular matrix and initiate wound healing and scarring. This fibrotic response is characterized by widespread molecular changes in activated resident fibroblasts that can be induced by TGFβ1, a multifunctional cytokine that is released by local and infiltrating cells.

To identify key markers of this transition we assessed and ranked global RNA expression differences via transcriptome sequencing in atrial fibroblasts derived from 80 individuals before and 24 hours after TGFβ1 treatment. As discussed in Example 1, IL-11 expression was significantly upregulated in activated fibroblasts and we showed for the first time that the IL-11 transcriptional response is higher than the transcriptional response of all other individual genes regulated in fibrosis (Figure 4a). Comparison of the IL-11 expression level in our model system to various human tissues indicated that high IL-11 levels were also very specific for the fibrotic response (Figure 4b), making it an ideal marker to assess the extent of fibrosis in the human body.

To further assess whether IL-11 acts as an autocrine signaling factor that drives fibrosis, we confirmed that an upregulation of IL-11 RNA (Figure 5a) lead to an increase in IL-11 secretion (Figure 5b) from atrial fibroblasts. Incubation of fibroblasts with IL-11 did not increase IL-11 RNA expression (Figure 5c), but lead to an increase in IL-11 secretion from the cells (Figure 5d). This shows that IL-11 is having an autocrine effect on fibroblasts that regulates the production of IL-11 protein at the translational level.

We then incubated atrial fibroblasts with TGFβ1, recombinant IL-11 or TGFβ1 and a neutralising anti- human IL-11 monoclonal antibody (Monoclonal Mouse IgG_{2A}; Clone #22626; Catalog No. MAB218; R&D Systems, MN, USA) and monitored cell proliferation, myofibroblast generation as well as periostin expression at the protein level. We observed an increase in activated fibroblasts (αSMA-positive cells), periostin production and cell proliferation at a similar level for both TGFβ1 and IL-11 stimulated fibroblasts. In addition to its pro-fibrotic function, IL-11 was also found to play a critical role in the TGFβ1 fibrosis itself. The pro-fibrotic effect of TGFβ1 was inhibited when we neutralized IL-11 with the antibody (Figures 6a-c). The same pattern was observed when we monitored the secretion of fibrosis markers such as IL6, MMP2 and TIMP1 (Figures 6d-f).

We then monitored the deposition of collagen, the pathognomonic hallmark of the fibrotic response, using a number of assays across several regulatory levels of gene expression. TGFβ1 was found to increase intracellular collagen (Figure 7a), secreted collagen (Figure 7b) as well as collagen RNA levels (Figure 7c) as expected. The response to IL-11 was only observed at the protein level (Figure 7a,b) and not on the RNA level (Figure 7c). Stimulation with TGFβ1 in parallel to inhibiting IL-11 led to an increase in collagen RNA but this TGFβ1-driven effect was not forwarded to the protein level.

To establish further the central role of IL-11 in fibrosis downstream of multiple pro-fibrotic stimuli, we assessed IL-11 expression across fibroblast populations derived from four different tissues in response to TGFβ1 (Figure 8a), ET-1, (Figure 8b) and PDGF (Figure 8c). We also administered recombinant IL-11 systemically to C57BL/6 mice and monitored collagen and αSMA expression. Collagen production was increased across kidney, heart and liver (Figure 8d) and we also detected more activated fibroblasts in the heart and kidney, indicated by higher αSMA protein levels (Figure 8e).

Our findings demonstrate a novel and central role for IL-11 in fibrosis and, most importantly, show that IL-11 is downstream of the key pro-fibrotic stimuli across several tissues. These results show that IL-11 is required for TGFβ1 to proceed from transcriptional regulation to protein translation. Inhibition of IL-11 stalls the pro-fibrotic effect of TGFβ1 on the transcriptome (Figure 9).

Example 3: Anti-IL-11 antibodies inhibit pro-fibrotic stimuli

In experiments similar to those described in respect of Figure 3c, atrial fibroblasts were exposed to other pro-fibrotic stimuli in the form of angiotensin II (ANG 2), platelet-derived growth factor (PDGF) and endothelin 1 (ET-1), and collagen production was measured.

In addition to induction of IL-11 mRNA expression, each of ANG2, PDGF and ET-1 induced IL-11 protein expression. Inhibition of IL-11 with a neutralising anti- human IL-11 monoclonal antibody (Monoclonal Mouse IgG_{2A}; Clone #22626; Catalog No. MAB218; R&D Systems, MN, USA) blocked the pro-fibrotic effect of each of these pro-fibrotic stimuli (Figure 10) indicating IL-11 to be the central effector of the major pro-fibrotic stimuli (TGFβ1, ANG2, PDGF and ET-1).

Example 4: IL-11R knockdown

HEK cells were transfected (24h) with non-targeting (NT) siRNA or one of four different siRNAs against the IL11RA1 receptor (siRNAs 5-8; Figure 14; SEQ ID NOs 15 to 18). RNA was extracted and assayed for IL11RA1 mRNA expression by qPCR. Data are shown in Figure 15 as mRNA expression levels relative to the control (NT).

Example 5: A role for IL-11 in fibrosis

5.1 IL-11 is upregulated in fibrosis

To understand the molecular processes underlying the transition of fibroblasts to activated myofibroblasts, atrial tissue was obtained from more than 200 patients that underwent cardiac bypass surgery at the National Heart Centre Singapore. Cells were cultured *in vitro* at low passage (passage <4), and either not stimulated or stimulated with TGFβ1 for 24h. We subsequently performed high-throughput RNA sequencing (RNA-seq) analysis of unstimulated fibroblasts and cells stimulated with the prototypic pro-fibrotic stimulus TGFβ1 across 160 individuals; average read depth was ~70M reads per sample (paired-end 100bp; Figure 16).

To ensure the purity of the atrial fibroblast cell cultures, we analysed expression of endothelial cell, cardiomyocyte and fibroblast cell type marker genes from the atrium (Hsu et al., 2012 Circulation Cardiovasc Genetics 5, 327–335) in the RNA-seq dataset.

The results are shown in Figures 17A to 17E, and confirm the purity of the atrial fibroblast cultures.

Gene expression was assessed by RNA-seq of the tissue of origin (human atrial tissues samples, n=8) and primary, unstimulated fibroblast cultures. No/very low expression of the endothelial cell marker PECAM1 (Figure 17A), and the cardiomyocyte markers MYH6 (Figure 17B) and TNNT2 (Figure 17C) was detected in the fibroblast cell culture samples. Markers for fibroblasts COL1A2 (Figure 17D) and ACTA2 (Figure 17E) were highly expressed compared to the tissue of origin.

Next, the RNA-seq data was analysed to identify genes whose expression was increased or decreased upon stimulation with TGFβ1, and this information was integrated with the large RNA-seq dataset across 35+ human tissues provided by the GTEx project (The GTEx Consortium, 2015 Science 348, 648–660). This enabled the identification of gene expression signatures that were specific to the fibroblast-myofibroblast transition.

The results are shown in Figures 18A to 18E. Across the 10000+ genes expressed in the fibroblasts, IL-11 was the most strongly upregulated gene in response to stimulation with TGFβ1, and on average across the 160 individuals was upregulated more than 10-fold (Figure 18A).

Upregulation of IL-11 expression was confirmed by ELISA analysis of the cell culture supernatant of TGFβ1 stimulated fibroblasts (Figure 18C). As compared to the level of expression level of IL-11 in other tissues of healthy individuals, this response was observed to be highly specific to activated fibroblasts (Figure 18D). Various fold changes of IL-11 RNA expression were also confirmed by qPCR analysis (Figure 18E).

Next, fibroblasts were cultured *in vitro* and stimulated with several other known pro-fibrotic factors: ET-1, ANGII, PDGF, OSM and IL-13, and also with human recombinant IL-11. For analysing upregulation of IL-11 produced in response to stimulation with IL-11, it was confirmed that the ELISA was only able to detect native IL-11 secreted from cells and does not detect recombinant IL-11 used for the stimulations (Figure 19B).

The results are shown in Figure 19A. Each factor was found to significantly induce IL-11 secretion from fibroblasts. IL-11 is shown to act in an autocrine loop in fibroblasts, which can result in an upregulation of IL-11 protein as much as 100-fold after 72 hours (Figure 19D).

Interestingly, this autocrine loop for IL-11 is similar to the autocrine production of IL-6. IL-6 is from the same cytokine family and also signals via the gp130 receptor (Garbers and Scheller, 2013 Biol Chem 394, 1145–1161), which is proposed to ensure the continued survival and growth of lung and breast cancer cells (Grivennikov and Karin, 2008 Cancer Cell 13, 7–9).

No increase in IL-11 RNA level was detected in response to stimulation with IL-11 (Figure 19D). Unlike TGFβ1, which increases IL-11 expression at both the RNA and protein level, therefore IL-11 seems to upregulate IL-11 expression only at the post-transcriptional level.

5.2 IL-11 has a profibrotic role in fibrosis of heart tissue

To explore whether the autocrine production of IL-11 is pro- or anti-fibrotic, fibroblasts were cultured *in vitro* with recombinant IL-11, and the fraction of myofibroblasts (αSMA-positive cells) and extracellular matrix production was analysed.

The expression of αSMA, collagen and periostin was monitored with the Operetta High-Content Imaging System in an automated, high-throughput fashion. In parallel, secretion of fibrosis marker proteins such as MMP2, TIMP1 and IL-6 was analysed by ELISA assays, and the levels of collagen were confirmed by calorimetric Sirius Red analysis of the cell culture supernatant.

Briefly, atrial fibroblasts derived from 3 individuals were incubated in 2 wells each for 24h without stimulation, with TGFβ1 (5 ng/ml), or with IL-11 (5 ng/ml). Following incubation, cells were stained to analyse α-SMA content to estimate the fraction of myofibroblasts, and for collagen and periostin to estimate ECM production. Fluorescence was measured in 7 fields per well. The supernatant of 2 wells per individual was also assessed for collagen content by Sirius Red staining. The signal was normalized to the control group without stimulation. Secretion of the fibrosis markers IL-6, TIMP1 and MMP2 was analysed via ELISA.

The results are shown in Figures 20A to 20F. TGFβ1 activated fibroblasts and increased ECM production (Figure 20A). Unexpectedly, and in contrast with the anti-fibrotic role described for IL-11 in heart tissue in the scientific literature, recombinant IL-11 caused an increase in the fraction of myofibroblasts in fibroblast cultures, and also promoted the production of extracellular matrix proteins collagen and periostin to the same extent as TGFβ1 (Figure 20A). Both of IL-11 and TGFβ1 cytokines also significantly increased the secretion of pro-fibrotic markers IL-6, TIMP1 and MMP2 (Figures 20B to 20E), and to a similar level.

The inventors hypothesized that the contradiction between the present finding that IL-11 is profibrotic in heart tissue and the antifibrotic role described in the literature might be related to the use of human IL-11 in rodents in those previous studies (Obana et al., 2010, 2012; Stangou et al., 2011; Trepicchio and Dorner, 1998).

To investigate this hypothesis, serial dilutions of both human and mouse IL-11 were performed, and the activation of human atrial fibroblasts was monitored (Figure 20F). No activation of fibroblasts was observed at low concentrations of human IL-11 on mouse cells, suggesting that previous insights into IL-11 function may in part be due to IL-11-non-specific observations.

5.3 IL-11 has a profibrotic role in fibrosis of a variety of tissues

To test whether the profibrotic action of IL-11 was specific to atrial fibroblasts, human fibroblasts derived from several different tissues (heart, lung, skin, kidney and liver) were cultured *in vitro*, stimulated with human IL-11, and fibroblast activation and ECM production was analysed as described above. Increased fibroblast activation and production of ECM was observed as compared to non-stimulated cultures in fibroblasts derived from each of the tissues analysed.

5.3.1 Liver fibrosis

To test whether IL-11 signalling is important in liver fibrosis, human primary liver fibroblasts (Cell Biologics, Cat#: H-6019) were cultured at low passage in wells of 96-well plates and either not stimulated, stimulated with TGFβ1 (5ng/ml, 24h), IL-11 (5 ng/ml, 24h) or incubated with both TGFβ1 (5 ng/ml) and a neutralising IL-11 antibody (2 µg/ml), or TGFβ1 (5 ng/ml) and an Isotype control antibody. Fibroblast activation (αSMA positive

cells), cell proliferation (EdU positive cells) and ECM production (periostin and collagen) was analysed using the Operetta platform.

The results of the experiments with primary human liver fibroblasts are shown in Figures 38A to 38D. IL-11 was found to activate liver fibroblasts, and IL-11 signalling was found to be necessary for the profibrotic action of TGFβ1 in liver fibroblasts. Both activation and proliferation of fibroblasts was inhibited by neutralising anti-IL-11 antibody.

5.3.2 Skin fibrosis

To test whether IL-11 signalling is important in skin fibrosis, primary mouse skin fibroblasts were cultured at low passage in wells of 96-well plates and either not stimulated, stimulated with TGFβ1 (5ng/ml, 24h) or incubated for 24h with both TGFβ1 (5 ng/ml) and a neutralising IL-11 antibody (2 µg/ml). Fibroblast activation (αSMA positive cells) was then analysed using the Operetta platform.

The results are shown in Figure 39. TGFβ1-mediated activation of skin fibroblasts was inhibited by neutralising anti-IL-11 antibody.

5.3.3 Fibrosis in multiple organs

Next, mouse recombinant IL-11 was injected (100µg/kg, 3 days/week, 28 days) into mice to test whether IL-11 can drive global tissue fibrosis *in vivo*.

The results are shown in Figure 21. Compared to injection of AngII (a cytokine that causes an elevation in blood pressure and hypertrophy of the heart), IL-11 also increased the heart weight but also kidney, lung and liver weight indexed to body weight (Figure 21B). Assessing collagen content in these issues by hydroxyproline assay revealed an upregulation of collagen production in these tissues, indicating fibrosis as the likely cause for the increase in organ weight (Figure 6C). Expression of fibrosis marker genes ACTA2 (= αSMA), Col1a1, Col3a1, Fn1, Mmp2 and Timp1 was also detected by qPCR analysis of RNA isolated from heart, kidney, lung and liver tissues of these animals.

Example 6: Therapeutic potential of IL-11/IL-11R antagonism

6.1 Inhibition of the fibrotic response using neutralising antagonists of IL-11/IL-11R

Next it was investigated whether the autocrine loop of IL-11 secretion was required for the pro-fibrotic effect of TGFβ1 on fibroblasts.

IL-11 was inhibited using a commercially available neutralizing antibody (Monoclonal Mouse IgG2A; Clone #22626; Catalog No. MAB218; R&D Systems, MN, USA). Fibroblasts were treated with TGFβ1 in the presence or absence of the antibody, and fibroblast activation, the proportion of proliferating cells and ECM production and markers of the fibrotic response were measured.

Briefly, atrial fibroblasts derived from 3 individuals were incubated for 24h with TGFβ1 (5 ng/ml) or TGFβ1 in the presence of neutralising anti-IL-11 antibody or isotype control antibody. Following incubation, cells were stained for αSMA to determine the fraction of myofibroblasts, the proportion of proliferating cells was determined by analysing the cells for EdU incorporation, and periostin was measured to determine ECM production. Fluorescence was measured with the Operetta platform for 14 fields across 2 wells for each individual. Secretion of the fibrosis markers IL-6, TIMP1 and MMP2 was also analysed by ELISA. Fluorescence was normalized to the control group without stimulation.

The results are shown in Figures 22A to 22F. IL-11 inhibition was found to ameliorate TGFβ1-induced fibrosis, and it was shown that IL-11 is essential for the pro-fibrotic effect of TGFβ1. Inhibition of IL-11 was found to 'rescue' the TGFβ1 phenotype at the protein level.

Collagen production was also analysed. Cardiac fibroblasts derived from 3 individuals were incubated for 24h with TGFβ1 (5 ng/ml) or TGFβ1 and a neutralizing IL-11 antibody. Following incubation the cells were stained for collagen using the Operetta assay and florescence was quantified as described above. Secreted collagen levels in the cell culture supernatant were assessed by Sirius Red staining.

The results are shown in Figures 23A and 23B, and confirm the anti-fibrotic effect of inhibition of IL-11 using a neutralising antibody.

Next, the ability of several other IL-11/IL-11R antagonists to inhibit fibrosis was analysed *in vitro* using the atrial fibroblast, TGFβ1-induced myofibroblast transition assay described herein above.

Briefly, human atrial fibroblasts cells were cultured *in vitro*, stimulated for 24h with TGFβ1 (5 ng/ml) or left unstimulated, in the presence/absence of: (i) neutralising anti-IL-11

antibody, (ii) a IL-11RA-gp130 fusion protein (iii) neutralising anti-IL-11RA antibody, (iv) treatment with siRNA directed against IL-11 or (v) treatment with siRNA directed against IL-11RA. The proportion of activated fibroblasts (myofibroblasts) was analysed by evaluating αSMA content as described above.

The results are shown in Figure 24. Each of the antagonists of IL-11/IL-11R signalling was found to be able to abrogate TGFβ1-mediated profibrotic response.

Example 7: *In vivo* confirmation of a profibrotic role for IL-11/IL-11R signalling

7.1 *In vitro* studies using cells derived from IL-11RA gene knock-out mice

All mice were bred and housed in the same room and provided food and water ad libitum. Mice lacking functional alleles for IL-11Rα (IL-11RA1 KO mice) were on C57Bl/6 genetic background. Mice were of 9-11 weeks of age and the weight of animals did not differ significantly.

To further confirm the anti-fibrotic effect of inhibition of IL-11/IL-11R signalling, primary fibroblasts were generated from IL-11RA gene knock-out mice and incubated with primary fibroblast cells harvested from IL-11RA+/+ (i.e. wildtype), IL-11RA+/- (i.e. heterozygous knockout) and IL-11RA-/- (i.e. homozygous knockout) animals with TGFβ1, IL-11 or AngII. Activation and proliferation of fibroblasts and ECM production was analysed.

Fibroblasts derived from IL-11RA+/+, IL-11RA+/- and IL-11RA-/- mice were incubated for 24 hours with TGFβ1, IL-11 or AngII (5 ng/ml). Following incubation, cells were stained for αSMA content to estimate the fraction of myofibroblasts, for EdU to identify the fraction of proliferating cells, and for collagen and periostin to estimate ECM production. Fluorescence was measured using the Operetta platform.

The results are shown in Figures 25A to 25D. IL-11RA-/- mice were found not to respond to pro-fibrotic stimuli. These results suggested that IL-11 signalling is also required for AngII-induced fibrosis.

Next, it was investigated whether this was also true for other pro-fibrotic cytokines. Briefly, fibroblasts were cultured *in vitro* in the presence/absence of various different pro-fibrotic factors (ANG2, ET-1 or PDGF), and in the presence/absence of neutralising anti-

IL-11 antibody or pan anti-TGFβ antibody. After 24 hours, collagen production by the cells was determined by analysis using the Operetta system as described above, and myofibroblast generation was determined by analysis of αSMA expression as described above.

The results are shown in Figures 26A and 26B. IL-11 was found to be required for fibrosis downstream of various profibrotic stimuli, and was thus identified as a central mediator of fibrosis induced by a variety of different profibrotic factors.

In a further experiment, the role of IL-11 signalling was investigated in lung fibrosis, using an *in vitro* scratch assay of migration of lung fibroblasts. In response to pro-fibrotic stimuli, fibroblasts are activated and migrate within the fibrotic niche in the body. The migration rate of cells is a measure of cell-cell and cell-matrix interactions and a model for wound healing *in vivo* (Liang et al., 2007; Nat Protoc. 2(2):329-33).

Fibroblasts derived from lung tissue from both wild type (WT) and also homozygous IL-11RA (-/-) knockout mice were grown at low passage on a plastic surface until they formed a uniform cell monolayer. A scratch was then created in the cell layer, and cell migration close to the scratch was monitored, either in the absence of stimulation, or in the presence of TGFβ1 or IL-11. Images captured at images at the two time points of immediately after creating the scratch and at 24h were used to determine the area covered by cells, and the rate of migration was compared between WT and KO fibroblasts. Cell migration (area in the scratch covered by cells after 24h) was normalized to the migration rate of WT cells without stimulus.

The results are shown in Figure 40. Lung fibroblasts derived from WT mice were shown to migrate faster in the presence of TGFβ1 and IL-11, indicating a pro-fibrotic effect of both cytokines in lung fibroblasts. Cells lacking IL-11 signalling derived from KO mice migrated more slowly as compared to WT cells. They also did not migrate faster in the presence of TGFβ1. The scratch assay revealed that lung fibroblasts lacking IL-11 signalling have a decrease cell migration rate both in the presence of TGFβ1 or IL-11, and at baseline. Thus, inhibition of IL-11 signalling is anti-fibrotic in the lung.

7.2 Heart fibrosis

The efficacy of IL-11 inhibition to treat fibrotic disorders was investigated *in vivo*. A mouse model for cardiac fibrosis, in which fibrosis is induced by treatment with AngII, was used to investigate whether IL-11RA -/- mice were protected from cardiac fibrosis.

Briefly, a pump was implanted, and wildtype (WT) IL-11RA(+/+) and knockout (KO) IL-11RA(-/-) mice were treated with AngII (2mg/kg/day) for 28 days. At the end of the experiment, collagen content was assessed in the atria of the mice using a calorimetric hydroxyproline-based assay kit, and the level of RNA expression of the markers or fibrosis Col1A2, αSMA (ACTA2) and fibronectin (Fn1) were analysed by qPCR.

The results are shown in Figures 27A to 27D. The IL-11RA-/- mice were found to be protected from the profibrotic effects of AngII.

7.3 Kidney fibrosis

A mouse model for kidney fibrosis was established in wildtype (WT) IL-11RA(+/+) and knockout (KO) IL-11RA(-/-) mice by intraperitoneal injection of folic acid (180mg/kg) in vehicle (0.3M $NaHCO_3$); control mice were administered vehicle alone. Kidneys were removed 28 days post-injection, weighed and either fixed in 10% neutral-buffered formalin for Masson's trichrome and Sirius staining or snap-frozen for collagen assay, RNA, and protein studies.

Total RNA was extracted from the snap-frozen kidney using Trizol reagent (Invitrogen) and Qiagen TissueLyzer method followed by RNeasy column (Qiagen) purification. The cDNA was prepared using iScriptTM cDNA synthesis kit, in which each reaction contained 1µg of total RNA, as per the manufacturer's instructions. Quantitative RT-PCR gene expression analysis was performed on triplicate samples with either TaqMan (Applied Biosystems) or fast SYBR green (Qiagen) technology using StepOnePlusTM (Applied Biosystem) over 40 cycles. Expression data were normalized to GAPDH mRNA expression level and we used the $2^{-\Delta\Delta Ct}$ method to calculate the fold-change. The snap-frozen kidneys were subjected to acid hydrolysis by heating in 6M HCl at a concentration of 50 mg/ml (95°C, 20 hours). The amount of total collagen in the hydrolysate was quantified based on the colorimetric detection of hydroxyproline using Quickzyme Total Collagen assay kit (Quickzyme Biosciences) as per the manufacturer's instructions.

The results of the analysis are shown in Figure 28. Folate-induced kidney fibrosis is shown to be dependent on IL-11 mediated signalling. A significant increase in collagen

content in kidney tissue was observed in IL-11RA+/+ mice, indicative of kidney fibrosis. No significant increase in collagen content was observed in IL-11RA -/- mice. Animals deficient for IL-11 signalling had significantly less collagen deposition in kidneys after toxic injury as compared to wild type animals.

7.4 Lung fibrosis

IL-11 is confirmed as a key mediator of fibrosis in the lung, skin and eye in further *in vivo* models using the IL-11RA -/- knockout mice. Schematics of the experiments are shown in Figures 29A to 29C.

To analyse pulmonary fibrosis, IL-11RA -/- mice and IL-11RA +/+ mice are treated by intratracheal administration of bleomycin on day 0 to establish a fibrotic response in the lung (pulmonary fibrosis). Fibrosis of the lung develops by 21 days, at which point animals are sacrificed and analysed for differences in fibrosis markers between animals with and without IL-11 signalling. IL-11RA -/- mice have a reduced fibrotic response in lung tissue as compared to IL-11RA +/+ mice, as evidenced by reduced expression of markers of fibrosis.

7.5 Skin fibrosis

To analyse fibrosis of the skin, IL-11RA -/- mice and IL-11RA +/+ mice are treated by subcutaneous administration of bleomycin on day 0 to establish a fibrotic response in the skin. Fibrosis of the skin develops by 28 days, at which point animals are sacrificed and analysed for differences in fibrosis markers between animals with and without IL-11 signalling. IL-11RA -/- mice have a reduced fibrotic response in skin tissue as compared to IL-11RA +/+ mice, as evidenced by reduced expression of markers of fibrosis.

7.6 Eye fibrosis

To analyse fibrosis in the eye, IL-11RA -/- mice and IL-11RA +/+ mice undergo trabeculectomy on day 0 to initiate a wound healing response in the eye. Fibrosis of the eye develops within 7 days. The fibrotic response is measured and compared between the IL-11RA -/- mice and IL-11RA +/+ mice. IL-11RA -/- mice have a reduced fibrotic response in eye tissue as compared to IL-11RA +/+ mice, as evidenced by reduced expression of markers of fibrosis.

7.7 Other tissues

The effect of IL-11RA knockout on fibrosis is also analysed in mouse models of fibrosis for other tissues, such as the liver, bowel, and is also analysed in a model relevant to multiorgan (i.e. systemic) fibrosis. The fibrotic response is measured and compared between the IL-11RA -/- mice and IL-11RA +/+ mice. IL-11RA -/- mice have a reduced fibrotic response as compared to IL-11RA +/+ mice, as evidenced by reduced expression of markers of fibrosis.

Example 8: Analysis of the molecular mechanisms underlying IL-11-mediated induction of fibrosis

The canonical mode of action of IL-11 is thought to be regulation of RNA expression via STAT3-mediated transcription (Zhu et al., 2015 PLoS ONE 10, e0126296), and also through activation of ERK.

STAT3 activation is observed following stimulation with IL-11. However, when fibroblasts are incubated with TGFβ1, only activation of the canonical SMAD pathway and ERK pathways is seen, and activation of STAT3 is not observed, even in spite of the fact that IL-11 is secreted in response to TGFβ1. Only ERK activation is common to both TGFβ1 and IL-11 signal transduction.

Cross-talk between TGFβ1 and IL-6 signalling has previously been described, wherein TGFβ1 blocks the activation of STAT3 by IL-6 (Walia et al., 2003 FASEB J. 17, 2130–2132). Given the close relationship between IL-6 and IL-11, similar cross-talk may be observed for IL-11 mediated signalling.

The inventors investigated by RNA-seq analysis whether regulation of RNA abundance was the underlying mechanism for the increased expression of fibrosis marker proteins in response to IL-11, which would suggest STAT3 as the underlying signalling pathway for IL-11 mediated profibrotic processes. Fibroblasts were incubated for 24 hours either without stimulus, or in the presence of TGFβ1, IL-11 or TGFβ1 and IL-11.

The results are shown in Figure 30A. TGFβ1 induced the expression of collagen, ACTA2 (αSMA) and other fibrosis marker at the RNA level. However, IL-11 did not regulate the expression of these genes, but a different set of genes.

Gene ontology analysis suggests that a pro-fibrotic effect in fibroblasts is driven by IL-11-regulated RNA expression. Both TGFβ1 and IL-11 regulate an almost completely different set of genes on the RNA level.

Whilst TGFβ1 increases IL-11 secretion, the target genes of IL-11 are not regulated when both TGFβ1 and IL-11 are present. This suggests that TGFβ1 upregulates IL-11 and simultaneously blocks the canonical IL-11-driven regulation of RNA expression via STAT3, similar to what is known about the interaction of TGFβ1 and IL-6 pathways (Walia et al., 2003 FASEB J. 17, 2130–2132).

We also analysed whether RNA expression differences induced by TGFβ1 are dependent on IL-11 signalling, by analysing changes in RNA expression in fibroblasts obtained from IL-11RA -/- mice as compared to IL-11RA +/+ mice. RNA expression regulated by TGFβ1 is still observed when IL-11RA knockout cells were stimulated with TGFβ1, and RNA levels of αSMA, collagen etc. were still upregulated in the absence of IL-11 signalling (in IL-11RA -/- fibroblasts). When the pro-fibrotic effect of IL-11 and the anti-fibrotic effect of IL-11 inhibition was investigated *in vitro*, reduced expression of markers of fibrosis was only observed at the protein level, not at the transcriptional level as determined by qPCR.

The activation of non-canonical pathways (e.g. ERK signal transduction) is known to be crucial for the pro-fibrotic action of TGFβ1 (Guo and Wang, 2008 Cell Res 19, 71–88). It is likely that non-canonical pathways are likely to be important for signalling for all known pro-fibrotic cytokines, and that IL-11 is a post-transcriptional regulator which is essential for fibrosis.

Example 9: Human anti-human IL-11 antibodies

Fully human anti-human IL-11 antibodies were developed via phage display.

Recombinant human IL-11 (Cat. No. Z03108-1) and recombinant murine IL-11 (Cat. No. Z03052-1) were obtained from GenScript (NJ, USA). Recombinant human IL-11 was expressed in CHO cells, both as an Fc-tagged version and a tag-free version. Tag-free murine IL-11 was expressed in HEK293 cells.

IL-11 bioactivity of recombinant human IL-11 and mouse IL-11 was confirmed by *in vitro* analysis using primary fibroblast cell cultures.

Recombinant, biotinylated human IL-11 and murine IL-11 were also prepared by biotinylation of the recombinant human IL-11 and murine IL-11 molecules, according to standard methods.

Antibodies capable of binding to both human IL-11 and murine IL-11 (i.e. cross-reactive antibodies) were identified by phage display using a human naïve library by panning using biotinylated and non-biotinylated recombinant human and murine IL-11, based on 16 different panning strategies.

The phage display identified 175 scFv binders, as 'first hits'. Sequence analysis of the CDR sequences from these 175 scFv identified 86 unique scFv.

The soluble scFv were produced by recombinant expression in E. coli, and analysed for their ability to bind to human IL-11 and murine IL-11 by ELISA. Briefly, the respective antigen was coated to wells of an ELISA plate, the cell culture supernatant containing the respective scFv was added at a 1:2 dilution, and binding was detected.

The results of the ELISA analysis revealed:

- 8 scFV capable of binding only to human IL-11;
- 6 scFv capable of binding to murine IL-11 only;
- 32 scFv displaying only weak binding to human/murine IL-11, with a high signal to noise ratio, and;
- 40 scFv having cross-reactivity for both human IL-11 and murine IL-11.

From these 86 scFV, 56 candidates were selected for further functional characterisation. For further analyses, the scFV were cloned into scFV-Fc format in E. coli.

The VH and VL sequences of the antibodies were cloned into expression vectors for the generation of scFv-Fc (human IgG1) antibodies. The vectors were transiently expressed in mammalian cells cultured in serum-free media, and isolated by protein A purification.

Example 10: Functional characterisation of human anti-human IL-11 antibodies

The antibodies described in Example 9 were analysed in *in vitro* assays for their ability to (i) inhibit human IL-11-mediated signalling, and (ii) inhibit mouse IL-11-mediated signalling. The affinity of the antibodies for human IL-11 was also analysed by ELISA.

10.1 Ability to inhibit human IL-11 mediated signalling

To investigate ability to neutralise human IL-11-mediated signalling, cardiac atrial human fibroblasts were cultured in wells of 96-well plates in the presence of TGFβ1 (5 ng/ml) for 24 hours, in the presence or absence of the anti-IL-11 antibodies. TGFβ1 promotes the expression of IL-11, which in turn drives the transistion of quiescent fibroblasts to activated, αSMA-positive fibroblasts. It has previously been shown that neutralising IL-11 prevents TGFβ1-induced transition to activated, αSMA-positive fibroblasts.

Expression of αSMA was analysed with the Operetta High-Content Imaging System in an automated high-throughput fashion.

In non-stimulated cultures, ~29.7% (= 1) of the fibroblasts were αSMA-positive, activated fibroblasts at the end of the 24 hour culture period, whilst ~52% (= 1.81) of fibroblasts were αSMA-positive in cultures that were stimulated with TGFβ1 in the absence of anti-IL-11 antibodies.

Anti-IL-11 antibodies (2 µg/ml) were added to fibroblast cultures that were stimulated with TGFβ1, and at the end of the 24 hour culture period, the percentage of αSMA-positive fibroblasts was determined. The percentages were normalised based on the percentage of αSMA-positive fibroblasts observed in cultures of fibroblasts which had not been stimulated with TGFβ1.

28 of the antibodies were demonstrated to be capable of neutralising signalling mediated by human IL-11.

A commercial monoclonal mouse anti-IL-11 antibody (Monoclonal Mouse IgG2A; Clone #22626; Catalog No. MAB218; R&D Systems, MN, USA) was also analysed for ability to inhibit signalling by human IL-11 in the experiments. This antibody was found to be able to reduce the percentage of activated fibroblasts to 28.3% (=0.99).

Several of the clones neutralised signalling by human IL-11 to a greater extent than the commercially available mouse anti-IL-11 antibody (industry standard.

10.2 Ability to inhibit mouse IL-11 mediated signalling

The ability of the human antibodies to inhibit mouse IL-11-mediated signalling was also investigated, following the same procedure as described in section 10.1 above, but using mouse dermal fibroblasts instead of human atrial fibroblasts.

After 24 hours in culture, about 31.8% (=1) of non-stimulated cells in culture were activated fibroblasts. Stimulation with TGFβ1 resulted in a ~2-fold increase in the percentage of activated fibroblasts (68.8% = 2.16) as compared to non-stimulated cultures.

The antibodies were demonstrated to be capable of neutralising signalling mediated by mouse IL-11. Monoclonal Mouse IgG2A clone #22626, catalog No. MAB218 anti-IL-11 antibody was also analysed for ability to inhibit signalling by mouse IL-11. This antibody was found to be able to reduce the percentage of activated fibroblasts to 39.4% (=1.24).

Several of the clones neutralised signalling by mouse IL-11 to a greater extent than the commercially available mouse anti-IL-11 antibody (industry standard).

10.3 Analysis of antibody affinity for human IL-11

The human anti-human IL-11 antibodies were analysed for their affinity of binding to human IL-11 by ELISA assay.

Recombinant human IL-11 was obtained from Genscript and Horseradish peroxidase (HRP)-conjugated anti-human IgG (Fc-specific) antibody was obtained from Sigma. Corning 96-well ELISA plates were obtained from Sigma. Pierce 3,3´,5,5´-tetramethylbenzidine (TMB) ELISA substrate kit was obtained from Life Technologies (0.4 g/mL TMB solution, 0.02 % hydrogen peroxide in citric acid buffer). Bovine serum albumin and sulphuric acid was obtained from Sigma. Wash buffer comprised 0.05% Tween-20 in phosphate buffered saline (PBS-T). ScFv-Fc antibodies were generated as described in above. Purified mouse and human IgG controls were purchased from Life Technologies. Tecan Infinite 200 PRO NanoQuant was used to measure absorbance.

Criss-cross serial dilution analysis was performed as described by Hornbeck et al., (2015) Curr Protoc Immunol 110, 2.1.1-23) to determine the optimal concentration of coating antigen, primary and secondary antibodies.

An indirect ELISA was performed to assess the binding affinity of primary ScFv-Fc antibodies at 50% of effective concentration (EC_{50}) as previously described (Unverdorben et al., (2016) MAbs 8, 120–128.). ELISA plates were coated with 1 µg/mL of recombinant human IL-11 overnight at 4°C and remaining binding sites were blocked with 2 % BSA in PBS. ScFv-Fc antibodies were diluted in 1% BSA in PBS, titrated to obtain working concentrations of 800, 200, 50, 12.5, 3.125, 0.78, 0.195, and 0.049 ng/mL, and incubated in duplicates for 2 hours at room temperature. Detection of antigen-antibody binding was performed with 15.625 ng/mL of HRP-conjugated anti-human IgG (Fc-specific) antibody. Following 2 hours of incubation with the detection antibody, 100 µl of TMB substrate was added for 15 mins and chromogenic reaction stopped with 100 µl of 2 M H_2SO_4. Absorbance reading was measured at 450 nm with reference wavelength correction at 570 nm. Data were fitted with GraphPad Prism software with log transformation of antibody concentrations followed by non-linear regression analysis with the asymmetrical (five-parameter) logistic dose-response curve to determine individual EC50 values.

The same materials and procedures as described above were performed to determine the affinity of binding for the murine monoclonal anti-IL-11 antibodies, with the exception that HRP-conjugated anti-mouse IgG (H&L) was used instead of HRP-conjugated anti-human IgG.

The same materials and procedures as described above were performed to determine the affinity of binding for the human monoclonal anti-IL-11 antibodies and murine monoclonal anti-IL-11 antibodies to recombinant murine IL-11 obtained from Genscript.

The results of the ELISA assays were used to determine EC_{50} values for the antibodies.

10.4 Ability to inhibit human IL-11 mediated signalling in a variety of tissues

Ability of the antibodies to neutralise IL-11-mediated signalling in fibroblasts obtained from a variety of different tissues is investigated, essentially as described in section 10.1 except that instead of cardiac atrial human fibroblasts, human fibroblasts derived from liver, lung, kidney, eye, skin, pancreas, spleen, bowel, brain, and bone marrow are used for the experiments.

Anti-IL-11 antibodies are demonstrated to be capable of neutralising signalling in fibroblasts derived from the various different tissues, as determined by observation of a relative decrease in the proportion of αSMA-positive fibroblasts at the end of the 24 h

culture period in the presence of the anti-IL-11 antibodies as compared to culture in the absence of the antibodies.

Example 11: Inhibition of fibrosis *in vivo* using anti-IL-11 antibodies

The therapeutic utility of the anti-human IL-11 antibodies is demonstrated in *in vivo* mouse models of fibrosis for various different tissues.

11.1 Heart fibrosis

A pump is implanted, and mice are treated with AngII (2mg/kg/day) for 28 days.

Neutralising anti-IL-11 antibodies, or control antibodies, are administered to different groups of mice by intravenous injection. At the end of the experiment, collagen content is assessed in the atria of the mice using a calorimetric hydroxyproline-based assay kit, and the level of RNA expression of the markers or fibrosis Col1A2, αSMA (ACTA2) and fibronectin (Fn1) were analysed by qPCR.

Mice treated with neutralising anti-IL-11 antibodies have a reduced fibrotic response in heart tissue as compared to mice treated with control antibodies, as evidenced by reduced expression of markers of fibrosis.

11.2 Kidney fibrosis

A mouse model for kidney fibrosis is established, in which fibrosis is induced by intraperitoneal injection of folic acid (180mg/kg) in vehicle (0.3M $NaHCO_3$); control mice were administered vehicle alone.

Neutralising anti-IL-11 antibodies, or control antibodies, are administered to different groups of mice by intravenous injection. Kidneys are removed at day 28, weighed and either fixed in 10% neutral-buffered formalin for Masson's trichrome and Sirius staining or snap-frozen for collagen assay, RNA, and protein studies.

Total RNA is extracted from the snap-frozen kidney using Trizol reagent (Invitrogen) and Qiagen TissueLyzer method followed by RNeasy column (Qiagen) purification. The cDNA is prepared using iScriptTM cDNA synthesis kit, in which each reaction contained 1µg of total RNA, as per the manufacturer's instructions. Quantitative RT-PCR gene expression analysis is performed on triplicate samples with either TaqMan (Applied Biosystems) or

fast SYBR green (Qiagen) technology using StepOnePlusTM (Applied Biosystem) over 40 cycles. Expression data are normalized to GAPDH mRNA expression level and the 2-ΔΔCt method is used to calculate the fold-change. The snap-frozen kidneys are subjected to acid hydrolysis by heating in 6M HCl at a concentration of 50 mg/ml (95°C,20 hours). The amount of total collagen in the hydrolysate is quantified based on the colorimetric detection of hydroxyproline using Quickzyme Total Collagen assay kit (Quickzyme Biosciences) as per the manufacturer's instructions.

Mice treated with neutralising anti-IL-11 antibodies have a reduced fibrotic response in kidney tissue as compared to mice treated with control antibodies, as evidenced by reduced expression of markers of fibrosis.

11.3 Lung fibrosis

Mice are treated by intratracheal administration of bleomycin on day 0 to establish a fibrotic response in the lung (pulmonary fibrosis).

Neutralising anti-IL-11 antibodies, or control antibodies, are administered to different groups of mice by intravenous injection. Mice are sacrificed at day 21, and analysed for differences in fibrosis markers.

Mice treated with neutralising anti-IL-11 antibodies have a reduced fibrotic response in lung tissue as compared to mice treated with control antibodies, as evidenced by reduced expression of markers of fibrosis.

11.4 Skin fibrosis

Mice are treated by subcutaneous administration of bleomycin on day 0 to establish a fibrotic response in the skin.

Neutralising anti-IL-11 antibodies, or control antibodies, are administered to different groups of mice by intravenous injection. Mice are sacrificed at day 21, and analysed for differences in fibrosis markers.

Mice treated with neutralising anti-IL-11 antibodies have a reduced fibrotic response in skin tissue as compared to mice treated with control antibodies, as evidenced by reduced expression of markers of fibrosis.

11.5 Eye fibrosis

Mice undergo trabeculectomy on day 0 to initiate a wound healing response in the eye.

Neutralising anti-IL-11 antibodies, or control antibodies, are administered to different groups of mice by intravenous injection, and fibrosis is monitored in the eye tissue.

Mice treated with neutralising anti-IL-11 antibodies have a reduced fibrotic response in eye tissue as compared to mice treated with control antibodies, as evidenced by reduced expression of markers of fibrosis.

11.6 Other tissues

The effect of treatment with neutralising anti-IL-11 antibodies on fibrosis is also analysed in mouse models of fibrosis for other tissues, such as the liver, kidney, bowel, and is also analysed in a model relevant to multiorgan (i.e. systemic) fibrosis.

Mice treated with neutralising anti-IL-11 antibodies have a reduced fibrotic response as compared to mice treated with control antibodies, as evidenced by reduced expression of markers of fibrosis.

Example 12: Anti-human IL-11Rα antibodies

Mouse monoclonal antibodies directed against human IL-11Rα protein were generated as follows.

cDNA encoding the amino acid for human IL-11Rα was cloned into expression plasmids (Aldevron GmbH, Freiburg, Germany).

Mice were immunised by intradermal application of DNA-coated gold-particles using a hand-held device for particle-bombardment ("gene gun"). Serum samples were collected from mice after a series of immunisations, and tested in flow cytometry on HEK cells which had been transiently transfected with human IL-11Rα expression plasmids (cell surface expression of human IL-11Rα by transiently transfected HEK cells was confirmed with anti-tag antibodies recognising a tag added to the N-terminus of the IL-11Rα protein).

Antibody-producing cells were isolated from the mice and fused with mouse myeloma cells (Ag8) according to standard procedures.

Hybridomas producing antibodies specific for IL-11Rα were identified by screening for ability to bind to IL-11Rα expressing HEK cells by flow cytometry.

Cell pellets of positive hybridoma cells were prepared using an RNA protection agent (RNAlater, cat. #AM7020 by ThermoFisher Scientific) and further processed for sequencing of the variable domains of the antibodies.

Sequencing was performed using BigDye® Terminator v3.1 Cycle Sequencing kit (Life Technologies®) according to the manufacturer's instructions. All data was collected using a 3730xl DNA Analyzer system and Unified Data Collection software (Life Technologies®). Sequence assembly was performed using CodonCode Aligner (CodonCode Corporation). Mixed base calls were resolved by automatically assigning the most prevalent base call to the mixed base calls. Prevalence was determined by both frequency of a base call and the individual quality of the base calls.

In total, 17 mouse monoclonal anti-human IL-11Rα antibody clones were generated.

Example 13: Functional characterisation of anti-human IL-11Rα antibodies

13.1 Ability to inhibit human IL-11/IL-11R mediated signalling

To investigate the ability of the anti-IL-11Rα antibodies to neutralise human IL-11/IL-11R mediated signalling, cardiac atrial human fibroblasts were cultured in wells of 96-well plates in the presence of TGFβ1 (5 ng/ml) for 24 hours, in the presence or absence of the anti-IL-11Rα antibodies. This profibrotic stimulus promotes the expression of IL-11, which in turn drives the transistion of quiescent fibroblasts to activated, αSMA-positive fibroblasts. It has previously been shown that neutralising IL-11 prevents TGFβ1-induced transition to activated, αSMA-positive fibroblasts.

Anti-IL-11Rα antibodies (2 µg/ml) were added to fibroblast cultures that were stimulated with TGFβ1, and at the end of the 24 hour culture period, the percentage of αSMA-positive fibroblasts was determined. The percentages were normalised based on the percentage of αSMA-positive fibroblasts observed in cultures of fibroblasts which had not been stimulated with TGFβ1.

Expression of αSMA was analysed with the Operetta High-Content Imaging System in an automated high-throughput fashion.

Stimulation with TGFβ1 resulted in a 1.58 fold increase in the number of αSMA-positive, activated fibroblasts at the end of the 24 hour culture period in the absence of anti-IL-11Rα antibodies.

A commercial monoclonal mouse anti-IL-11 antibody (Monoclonal Mouse IgG2A; Clone #22626; Catalog No. MAB218; R&D Systems, MN, USA) was included as a control. This antibody was found to be able to reduce the percentage of activated fibroblasts to 0.89 fold of the percentage of activated fibroblasts in unstimulated cultures (i.e. in the absence of stimulation with TGFβ1).

The anti-IL-11Rα antibodies were found to be able to inhibit IL-11/IL-11R signalling in human fibroblasts, and several were able to inhibit IL-11/IL-11R signalling to a greater extent than the monoclonal mouse anti-IL-11 antibody.

13.2 Ability to inhibit mouse IL-11 mediated signalling

The ability of the anti-IL-11Rα antibodies to inhibit mouse IL-11-mediated signalling was also investigated, following the same procedure as described in section 13.1 above, but using mouse atrial fibroblasts instead of human atrial fibroblasts.

Stimulation with TGFβ1 resulted in a 2.24 fold increase in the number of αSMA-positive, activated fibroblasts at the end of the 24 hour culture period in the absence of anti-IL-11Rα antibodies.

The commercial monoclonal mouse anti-IL-11 antibody (Monoclonal Mouse IgG2A; Clone #22626; Catalog No. MAB218; R&D Systems, MN, USA) was included as a control. This antibody was found to be able to reduce the percentage of activated fibroblasts to 1.44 fold of the percentage of activated fibroblasts in unstimulated cultures (i.e. in the absence of stimulation with TGFβ1).

The anti-IL-11Rα antibodies were found to be able to inhibit IL-11/IL-11R signalling in mouse fibroblasts, and several were able to inhibit IL-11/IL-11R signalling to a greater extent than the monoclonal mouse anti-IL-11 antibody.

13.3 Screening for ability to bind IL-11Rα

The mouse hybridomas producing anti-human IL-11Rα antibodies were sub-cloned, and cell culture supernatant from the subcloned hybridomas was analysed by "mix-and-measure" iQue assay for (i) ability to bind to human IL-11Rα, and (ii) cross reactivity for antigen other than IL-11Rα.

Briefly, labelled control cells (not expressing IL-11Rα at the cell surface) and unlabelled target cells expressing human IL-11Rα at their surface (following transient transfection with a plasmid encoding a FLAG-tagged human IL-11Rα) were mixed together with the cell culture supernatant (containing mouse-anti-IL-11Rα antibodies) and secondary detection antibodies (fluorescently-labelled anti-mouse IgG antibody).

The cells were then analysed using the HTFC Screening System (iQue) for the two labels (i.e. the cell label and the label on the secondary antibody). Detection of the secondary antibody on the unlabelled, IL-11Rα expressing cells indicated ability of the mouse-anti-IL-11Rα antibodies to bind to IL-11Rα. Detection of the secondary antibody on the labelled, control cells indicated cross-reactivity of the mouse-anti-IL-11Rα antibodies for target other than IL-11Rα.

As a positive control condition, labelled and unlabelled cells were incubated with a mouse anti-FLAG tag antibody as the primary antibody.

The majority of the subcloned hybridomas expressed antibody which was able to bind to human IL-11Rα, and which recognised this target with high specificity.

13.4 Analysis of antibody affinity for human IL-11Rα

The anti-human IL-11Rα antibodies are analysed for their affinity of binding to human IL-11Rα by ELISA assay.

Recombinant human IL-11Rα is obtained from Genscript and Horseradish peroxidase (HRP)-conjugated anti-human IgG (Fc-specific) antibody is obtained from Sigma. Corning 96-well ELISA plates are obtained from Sigma. Pierce 3,3´,5,5´-tetramethylbenzidine (TMB) ELISA substrate kit is obtained from Life Technologies (0.4 g/mL TMB solution, 0.02 % hydrogen peroxide in citric acid buffer). Bovine serum albumin and sulphuric acid is obtained from Sigma. Wash buffer comprises 0.05% Tween-20 in phosphate buffered saline (PBS-T). Purified IgG controls are purchased from Life Technologies. Tecan Infinite 200 PRO NanoQuant is used to measure absorbance.

Criss-cross serial dilution analysis was performed as described by Hornbeck et al., (2015) Curr Protoc Immunol 110, 2.1.1-23) to determine the optimal concentration of coating antigen, primary and secondary antibodies.

An indirect ELISA is performed to assess the binding affinity of the mouse anti-IL-11Rα antibodies at 50% of effective concentration (EC_{50}) as previously described (Unverdorben et al., (2016) MAbs 8, 120–128.). ELISA plates are coated with 1 µg/mL of recombinant human IL-11Rα overnight at 4°C, and remaining binding sites are blocked with 2 % BSA in PBS. The antibodies are diluted in 1% BSA in PBS, titrated to obtain working concentrations of 800, 200, 50, 12.5, 3.125, 0.78, 0.195, and 0.049 ng/mL, and incubated in duplicates for 2 hours at room temperature. Detection of antigen-antibody binding is performed with 15.625 ng/mL of HRP-conjugated anti-mouse IgG antibody. Following 2 hours of incubation with the detection antibody, 100 µl of TMB substrate is added for 15 mins and chromogenic reaction stopped with 100 µl of 2 M H_2SO_4. Absorbance reading is measured at 450 nm with reference wavelength correction at 570 nm. Data are fitted with GraphPad Prism software with log transformation of antibody concentrations followed by non-linear regression analysis with the asymmetrical (five-parameter) logistic dose-response curve to determine individual EC50 values.

13.5 Ability to inhibit human IL-11/IL-11R signalling in a variety of tissues

Ability of the antibodies to neutralise IL-11/IL-11R signalling in fibroblasts obtained from a variety of different tissues is investigated, essentially as described in section 13.1 except that instead of cardiac atrial human fibroblasts, human fibroblasts derived from liver, lung, kidney, eye, skin, pancreas, spleen, bowel, brain, and bone marrow are used for the experiments.

Anti-IL-11Rα antibodies are demonstrated to be capable of neutralising IL-11/IL-11R signalling in fibroblasts derived from the various different tissues, as determined by observation of a relative decrease in the proportion of αSMA-positive fibroblasts at the end of the 24 h culture period in the presence of the anti-IL-11Rα antibodies as compared to culture in the absence of the antibodies.

Example 14: Inhibition of fibrosis *in vivo* using anti-IL-11Rα antibodies

The therapeutic utility of the anti-human IL-11Rα antibodies is demonstrated *in vivo* in mouse models of fibrosis for various different tissues.

14.1 Heart fibrosis

A pump is implanted, and mice are treated with AngII (2mg/kg/day) for 28 days.

Neutralising anti-IL-11Rα antibodies, or control antibodies, are administered to different groups of mice by intravenous injection. At the end of the experiment, collagen content is assessed in the atria of the mice using a calorimetric hydroxyproline-based assay kit, and the level of RNA expression of the markers or fibrosis Col1A2, αSMA (ACTA2) and fibronectin (Fn1) were analysed by qPCR.

Mice treated with neutralising anti-IL-11Rα antibodies have a reduced fibrotic response in heart tissue as compared to mice treated with control antibodies, as evidenced by reduced expression of markers of fibrosis.

14.2 Kidney fibrosis

A mouse model for kidney fibrosis is established, in which fibrosis is induced by intraperitoneal injection of folic acid (180mg/kg) in vehicle (0.3M $NaHCO_3$); control mice were administered vehicle alone.

Neutralising anti-IL-11Rα antibodies, or control antibodies, are administered to different groups of mice by intravenous injection. Kidneys are removed at day 28, weighed and either fixed in 10% neutral-buffered formalin for Masson's trichrome and Sirius staining or snap-frozen for collagen assay, RNA, and protein studies.

Total RNA is extracted from the snap-frozen kidney using Trizol reagent (Invitrogen) and Qiagen TissueLyzer method followed by RNeasy column (Qiagen) purification. The cDNA is prepared using iScriptTM cDNA synthesis kit, in which each reaction contained 1µg of total RNA, as per the manufacturer's instructions. Quantitative RT-PCR gene expression analysis is performed on triplicate samples with either TaqMan (Applied Biosystems) or fast SYBR green (Qiagen) technology using StepOnePlusTM (Applied Biosystem) over 40 cycles. Expression data are normalized to GAPDH mRNA expression level and the $2^{-\Delta\Delta Ct}$ method is used to calculate the fold-change. The snap-frozen kidneys are subjected to acid hydrolysis by heating in 6M HCl at a concentration of 50 mg/ml (95°C,20 hours). The amount of total collagen in the hydrolysate is quantified based on the colorimetric detection of hydroxyproline using Quickzyme Total Collagen assay kit (Quickzyme Biosciences) as per the manufacturer's instructions.

Mice treated with neutralising anti-IL-11Rα antibodies have a reduced fibrotic response in kidney tissue as compared to mice treated with control antibodies, as evidenced by reduced expression of markers of fibrosis.

14.3 Lung fibrosis

Mice are treated by intratracheal administration of bleomycin on day 0 to establish a fibrotic response in the lung (pulmonary fibrosis).

Neutralising anti-IL-11Rα antibodies, or control antibodies, are administered to different groups of mice by intravenous injection. Mice are sacrificed at day 21, and analysed for differences in fibrosis markers.

Mice treated with neutralising anti-IL-11Rα antibodies have a reduced fibrotic response in lung tissue as compared to mice treated with control antibodies, as evidenced by reduced expression of markers of fibrosis.

14.4 Skin fibrosis

Mice are treated by subcutaneous administration of bleomycin on day 0 to establish a fibrotic response in the skin.

Neutralising anti-IL-11Rα antibodies, or control antibodies, are administered to different groups of mice by intravenous injection. Mice are sacrificed at day 21, and analysed for differences in fibrosis markers.

Mice treated with neutralising anti-IL-11Rα antibodies have a reduced fibrotic response in skin tissue as compared to mice treated with control antibodies, as evidenced by reduced expression of markers of fibrosis.

14.5 Eye fibrosis

Mice undergo trabeculectomy on day 0 to initiate a wound healing response in the eye.

Neutralising anti-IL-11Rα antibodies, or control antibodies, are administered to different groups of mice by intravenous injection, and fibrosis is monitored in the eye tissue.

Mice treated with neutralising anti-IL-11Rα antibodies have a reduced fibrotic response in eye tissue as compared to mice treated with control antibodies, as evidenced by reduced expression of markers of fibrosis.

14.6 Other tissues

The effect of treatment with neutralising anti-IL-11Rα antibodies on fibrosis is also analysed in mouse models of fibrosis for other tissues, such as the liver, kidney, bowel, and is also analysed in a model relevant to multiorgan (i.e. systemic) fibrosis.

Mice treated with neutralising anti-IL-11Rα antibodies have a reduced fibrotic response as compared to mice treated with control antibodies, as evidenced by reduced expression of markers of fibrosis.

Example 15: Decoy IL-11 Receptors

15.1 Decoy IL-11 Receptor constructs

Decoy IL-11 Receptor molecules were designed and cloned into the pTT5 vector for recombinant expression in 293-6E cells.

Briefly, an insert for the plasmid comprising cDNA encoding the ligand binding domains D1, D2 and D3 of gp130 in-frame with cDNA encoding either a 50 amino acid or 33 amino acid linker region, followed by cDNA encoding the ligand binding domains D2 and D3 of human IL-11Rα, followed by cDNA encoding the FLAG tag. The cDNA insert incorporated a leader sequence, Kozak sequences at the 5' end, and included a 5' EcoRI restriction site and a 3' HindIII restriction site (downstream of a stop codon) for insertion into the pTT5 vector.

The two constructs encoding a decoy IL-11 receptor molecule having either a 50 amino acid or 33 amino acid sequence are respectively designated Decoy IL-11 Receptor 1 (D11R1) and Decoy IL-11 Receptor 2 (D11R2).

15.2 Decoy IL-11 Receptor expression and purification

The constructs were transfected into 293-6E cells for recombinant expression and purification.

293-6E cells were grown in serum-free FreeStyle™ 293 Expression Medium (Life Technologies, Carlsbad, CA, USA). Cells were maintained in Erlenmeyer Flasks (Corning Inc., Acton, MA) at 37°C with 5% CO_2 on an orbital shaker (VWR Scientific, Chester, PA).

One day before transfection, the cells were seeded at an appropriate density in Corning Erlenmeyer Flasks. On the day of transfection, DNA and transfection reagent were mixed at an optimal ratio and then added into the flask with cells ready for transfection. The recombinant plasmids encoding D11R1 and D11R2 were transiently transfected into suspension 293-6E cell cultures on two separate days.

Cell culture supernatants were collected on day 6 and used for purification. Briefly, cell culture broths were centrifuged and filtrated. 0.5 ml of resin was added to cell culture supernatants and incubated for 3-4 hours to capture the target protein.

After washing and elution with appropriate buffers, eluted fractions were analysed by SDS-PAGE and Western blot using Rabbit anti-FLAG polyclonal Ab (GenScript, Cat.No.A00170) to confirm expression of the FLAG-tagged decoy IL-11 receptor molecules.

The purified species were quantified and stored at -80°C.

Example 16: Functional characterisation of Decoy IL-11 Receptors

16.1 Ability to inhibit human IL-11 mediated signalling

To investigate ability to neutralise human IL-11-mediated signalling, cardiac atrial human fibroblasts were cultured in wells of 96-well plates in the presence of TGFβ1 (5 ng/ml) for 24 hours, in the presence or absence of various concentrations of D11R1 or D11R2.

TGFβ1 promotes the expression of IL-11, which in turn drives the transition of quiescent fibroblasts to activated, αSMA-positive fibroblasts. It has previously been shown that neutralising IL-11 prevents TGFβ1-induced transition to activated, αSMA-positive fibroblasts.

Expression of αSMA was analysed with the Operetta High-Content Imaging System in an automated high-throughput fashion.

D11R1 or D11R2 were added to fibroblast cultures that were stimulated with TGFβ1 at final concentrations of 5 ng/ml, 50 ng/ml and 500 ng/ml, and at the end of the 24 hour culture period, the percentage of αSMA-positive fibroblasts in the culture was determined.

Both D11R1 and D11R2 were demonstrated to be capable of neutralising signalling mediated by human IL-11 in a dose-dependent manner.

The results of the experiments are shown in Figures 32A and 32B. Both D11R1 and D11R2 were demonstrated to be capable of neutralising signalling mediated by human IL-11 in a dose-dependent manner.

The IC_{50} for the D11R1 and D11R2 molecules was determined to be ~1 nM.

16.2 Ability to inhibit mouse IL-11 mediated signalling

The ability of D11R1 and D11R2 to inhibit mouse IL-11-mediated signalling is investigated, following the same procedure as described in section 16.1 above, but using mouse dermal fibroblasts instead of human atrial fibroblasts.

D11R1 and D11R2 are demonstrated to be capable of neutralising IL-11/IL-11R signalling in mouse dermal fibroblasts, as determined by observation of a relative decrease in the proportion of αSMA-positive fibroblasts at the end of the 24 h culture period in the presence of D11R1 or D11R2 as compared to culture in the absence of the decoy IL-11 receptors.

16.3 Analysis of decoy IL-11 receptor affinity for IL-11

D11R1 and D11R2 are analysed for their affinity of binding to human IL-11 by ELISA assay.

Recombinant human IL-11 was obtained from Genscript and Horseradish peroxidase (HRP)-conjugated anti-FLAG antibody is obtained. Corning 96-well ELISA plates were obtained from Sigma. Pierce 3,3´,5,5´-tetramethylbenzidine (TMB) ELISA substrate kit was obtained from Life Technologies (0.4 g/mL TMB solution, 0.02 % hydrogen peroxide in citric acid buffer). Bovine serum albumin and sulphuric acid was obtained from Sigma. Wash buffer comprised 0.05% Tween-20 in phosphate buffered saline (PBS-T). Tecan Infinite 200 PRO NanoQuant is used to measure absorbance.

An indirect ELISA is performed to assess the binding affinity of D11R1 and D11R2 at 50% of effective concentration (EC_{50}) as previously described (Unverdorben et al., (2016) MAbs 8, 120–128.). ELISA plates are coated with 1 µg/mL of recombinant human IL-11 overnight at 4°C and remaining binding sites were blocked with 2 % BSA in PBS. D11R1 and D11R1 are diluted in 1% BSA in PBS, titrated to obtain working concentrations of 800, 200, 50, 12.5, 3.125, 0.78, 0.195, and 0.049 ng/mL, and incubated in duplicates for 2 hours at room temperature. Detection of antigen-decoy IL-11 receptor binding is performed with HRP-conjugated anti-FLAG antibody. Following 2 hours of incubation with the detection antibody, 100 µl of TMB substrate is added for 15 mins and chromogenic reaction stopped with 100 µl of 2 M H_2SO_4. Absorbance reading is measured at 450 nm with reference wavelength correction at 570 nm. Data are fitted with GraphPad Prism software with log transformation of decoy IL-11 receptor concentrations followed by non-linear regression analysis with the asymmetrical (five-parameter) logistic dose-response curve to determine EC50 values.

The same materials and procedures as described above were performed to determine the affinity of binding to recombinant murine IL-11 obtained from Genscript.

16.4 Ability to inhibit human IL-11 mediated signalling in a variety of tissues

Ability of the decoy IL-11 receptors D11R1 and D11R2 to neutralise IL-11-mediated signalling in fibroblasts obtained from a variety of different tissues is investigated, essentially as described in sections 18.1 except that instead of cardiac atrial human fibroblasts, human fibroblasts derived from liver, lung, kidney, eye, skin, pancreas, spleen, bowel, brain, and bone marrow are used for the experiments.

D11R1 and D11R2 are demonstrated to be capable of neutralising signalling in fibroblasts derived from the various different tissues, as determined by observation of a relative decrease in the proportion of αSMA-positive fibroblasts at the end of the 24 h culture period in the presence of the decoy IL-11 receptors as compared to culture in the absence of the decoy IL-11 receptors.

Example 17: Inhibition of fibrosis *in vivo* using decoy IL-11 receptors

The therapeutic utility of the decoy IL-11 receptors is demonstrated in *in vivo* mouse models of fibrosis for various different tissues.

17.1 Heart fibrosis

A pump is implanted, and mice are treated with AngII (2mg/kg/day) for 28 days.

Decoy IL-11 receptors D11R1 or D11R2 are administered to different groups of mice by intravenous injection. At the end of the experiment, collagen content is assessed in the atria of the mice using a calorimetric hydroxyproline-based assay kit, and the level of RNA expression of the markers or fibrosis Col1A2, αSMA (ACTA2) and fibronectin (Fn1) were analysed by qPCR.

Mice treated with decoy IL-11 receptors have a reduced fibrotic response in heart tissue as compared to untreated/vehicle treated controls, as evidenced by reduced expression of markers of fibrosis.

17.2 Kidney fibrosis

A mouse model for kidney fibrosis is established, in which fibrosis is induced by intraperitoneal injection of folic acid (180mg/kg) in vehicle (0.3M $NaHCO_3$); control mice were administered vehicle alone.

Decoy IL-11 receptors D11R1 or D11R2 are administered to different groups of mice by intravenous injection. Kidneys are removed at day 28, weighed and either fixed in 10% neutral-buffered formalin for Masson's trichrome and Sirius staining or snap-frozen for collagen assay, RNA, and protein studies.

Total RNA is extracted from the snap-frozen kidney using Trizol reagent (Invitrogen) and Qiagen TissueLyzer method followed by RNeasy column (Qiagen) purification. The cDNA is prepared using iScriptTM cDNA synthesis kit, in which each reaction contained 1µg of total RNA, as per the manufacturer's instructions. Quantitative RT-PCR gene expression analysis is performed on triplicate samples with either TaqMan (Applied Biosystems) or fast SYBR green (Qiagen) technology using StepOnePlusTM (Applied Biosystem) over 40 cycles. Expression data are normalized to GAPDH mRNA expression level and the 2-ΔΔCt method is used to calculate the fold-change. The snap-frozen kidneys are subjected to acid hydrolysis by heating in 6M HCl at a concentration of 50 mg/ml (95°C,20 hours). The amount of total collagen in the hydrolysate is quantified based on the colorimetric detection of hydroxyproline using Quickzyme Total Collagen assay kit (Quickzyme Biosciences) as per the manufacturer's instructions.

Mice treated with decoy IL-11 receptors have a reduced fibrotic response in kidney tissue as compared to untreated/vehicle treated controls, as evidenced by reduced expression of markers of fibrosis.

17.3 Lung fibrosis

Mice are treated by intratracheal administration of bleomycin on day 0 to establish a fibrotic response in the lung (pulmonary fibrosis).

Decoy IL-11 receptors D11R1 or D11R2 are administered to different groups of mice by intravenous injection. Mice are sacrificed at day 21, and analysed for differences in fibrosis markers.

Mice treated with decoy IL-11 receptors have a reduced fibrotic response in lung tissue as compared to untreated/vehicle treated controls, as evidenced by reduced expression of markers of fibrosis.

17.4 Skin fibrosis

Mice are treated by subcutaneous administration of bleomycin on day 0 to establish a fibrotic response in the skin.

Decoy IL-11 receptors D11R1 or D11R2 are administered to different groups of mice by intravenous injection. Mice are sacrificed at day 21, and analysed for differences in fibrosis markers.

Mice treated with decoy IL-11 receptors have a reduced fibrotic response in skin tissue as compared to untreated/vehicle treated controls, as evidenced by reduced expression of markers of fibrosis.

17.5 Eye fibrosis

Mice undergo trabeculectomy procedure as described in Example 7.6 above to initiate a wound healing response in the eye.

Decoy IL-11 receptors D11R1 or D11R2 are administered to different groups of mice by intravenous injection, and fibrosis is monitored in the eye tissue.

Mice treated with decoy IL-11 receptors have a reduced fibrotic response in eye tissue as compared to untreated/vehicle treated controls, as evidenced by reduced expression of markers of fibrosis.

17.6 Other tissues

The effect of treatment with decoy IL-11 receptors D11R1 or D11R2 on fibrosis is also analysed in mouse models of fibrosis for other tissues, such as the liver, kidney, bowel, and is also analysed in a model relevant to multiorgan (i.e. systemic) fibrosis.

The fibrotic response is measured and compared between mice treated with decoy IL-11 receptors and untreated mice, or vehicle treated controls. . Mice treated with decoy IL-11 receptors have a reduced fibrotic response as compared to untreated/vehicle treated controls, as evidenced by reduced expression of markers of fibrosis.

Example 18: Genetic biomarkers for IL-11 response

In addition to measuring IL-11 protein as a potential biomarker for fibrosis, we developed an assay that can predict IL-11 secretion status in humans. This assay could be used as a companion diagnostic in IL-11-related clinical trials.

We first generated RNA-seq data (Figure 16**Error! Reference source not found.**) and determined the genotype of 69 ethnically matched (Chinese) individuals in the cohort using a SNP array based on fluorescent probe hybridization supplied by Illumina (HumanOmniExpress 24).

We then performed genome-wide linkage eQTL analysis to assess whether Single Nucleotide Polymorphisms (SNPs) affect RNA transcript levels of IL-11 or IL-11RA in unstimulated fibroblasts, in TGFB1 stimulated (5ng/ml, 24h) fibroblasts. We also tested if the increase in IL-11 upon TGFβ1 stimulation (= response) was dependent on the genotype.

At first we quantified the read count for both IL-11 and IL-11RA in all individuals and transformed these counts using the variance stabilization (VST) approach of the DESeq2 method (Love et al., Genome Biology 2014 15:550). We then considered IL-11 and IL-11RA expression in unstimulated (VST_{unstim}) and stimulated (VST_{stim}) cells. To assess the increase in IL-11, we also computed the delta in expression as $VST_{stim} - VST_{unstim}$. We corrected the expression values using covariates such as RNA sequencing library batch, RNA RIN quality score, library concentration, library fragment size, age, gender before

analyses. SNP and transcript expression, or delta expression, pairs were analysed using the matrix eQTL approach (Andrey A. Shabalin., Bioinformatics 2012 May 15; 28(10): 1353-1358).

We did not observe variation in *cis* or *trans* that significantly affected IL-11 expression in unstimulated cells. However, we detected distant SNPs that regulated the expression in stimulated = fibrotic fibroblasts. These variants stratify the population between individuals that do express low levels of IL-11 and those that express high amounts of IL-11 in fibrosis. We also detected local and distal variants that predicted the increase in IL-11 expression in response to TGFβ1. These variants can be used to stratify individuals into high and low responders in fibrosis.

The SNPs identified are shown in Figures 33 to 35 and accompanying data is shown in Figures 36 and 37.

Claims:

1. An agent capable of inhibiting the action of Interleukin 11 (IL-11) for use in a method of treating or preventing fibrosis.

2. Use of an agent capable of inhibiting the action of Interleukin 11 (IL-11) in the manufacture of a medicament for use in a method of treating or preventing fibrosis.

3. A method of treating or preventing fibrosis, the method comprising administering to a subject in need of treatment a therapeutically effective amount of an agent capable of inhibiting the action of Interleukin 11 (IL-11).

4. The agent for use in a method of treating or preventing fibrosis according to claim 1, use according to claim 2 or method according to claim 3, wherein the agent is an agent capable of preventing or reducing the binding of IL-11 to an IL-11 receptor.

5. The agent for use in a method of treating or preventing fibrosis according to claim 1 or 4, use according to claim 2 or 4, or method according to claim 3 or 4, wherein the agent is an IL-11 binding agent.

6. The agent for use in a method of treating or preventing fibrosis, use or method according to claim 5, wherein the IL-11 binding agent is selected from the group consisting of: an antibody, polypeptide, peptide, oligonucleotide, aptamer or small molecule.

7. The agent for use in a method of treating or preventing fibrosis, use or method according to claim 5, wherein the IL-11 binding agent is an antibody.

8. The agent for use in a method of treating or preventing fibrosis, use or method according to claim 5, wherein the IL-11 binding agent is a decoy receptor.

9. The agent for use in a method of treating or preventing fibrosis according to claim 1 or 4, use according to claim 2 or 4, or method according to claim 3 or 4, wherein the agent is an IL-11 receptor (IL-11R) binding agent.

10. The agent for use in a method of treating or preventing fibrosis, use or method according to claim 9, wherein the IL-11R binding agent is selected from the group consisting of: an antibody, polypeptide, peptide, oligonucleotide, aptamer or small molecule.

11. The agent for use in a method of treating or preventing fibrosis, use or method according to claim 9, wherein the IL-11R binding agent is an antibody.

12. An agent capable of preventing or reducing the expression of Interleukin 11 (IL-11) or an Interleukin 11 receptor (IL-11R) for use in a method of treating or preventing fibrosis.

13. Use of an agent capable of preventing or reducing the expression of Interleukin 11 (IL-11) or an Interleukin 11 receptor (IL-11R) in the manufacture of a medicament for use in a method of treating or preventing fibrosis.

14. A method of treating or preventing fibrosis, the method comprising administering to a subject in need of treatment a therapeutically effective amount of an agent capable of preventing or reducing the expression of Interleukin 11 (IL-11) or an Interleukin 11 receptor (IL-11R).

15. The agent for use in a method of treating or preventing fibrosis according to claim 12, use according to claim 13 or method according to claim 14, wherein the agent is a small molecule or oligonucleotide.

16. The agent for use in a method of treating or preventing fibrosis, use or method according to any one of the preceding claims, wherein the fibrosis is fibrosis of the heart, liver, kidney or eye.

17. The agent for use in a method of treating or preventing fibrosis, use or method according to any one of the preceding claims, wherein the fibrosis is in the heart and is associated with dysfunction of the musculature or electrical properties of the heart, or thickening of the walls or valves of the heart.

18. The agent for use in a method of treating or preventing fibrosis, use or method according to any one of the preceding claims, wherein the fibrosis is in the liver and is associated with chronic liver disease or liver cirrhosis.

19. The agent for use in a method of treating or preventing fibrosis, use or method according to any one of the preceding claims, wherein the fibrosis is in the kidney and is associated with chronic kidney disease.

20. The agent for use in a method of treating or preventing fibrosis, use or method according to any one of the preceding claims, wherein the fibrosis is in the eye and is retinal fibrosis, epiretinal fibrosis, or subretinal fibrosis.

21. The agent for use in a method of treating or preventing fibrosis, use or method according to any one of the preceding claims, wherein the method of treating or preventing comprises administering said agent to a subject in which IL-11 or IL-11R expression is upregulated.

22. The agent for use in a method of treating or preventing fibrosis, use or method according to any one of the preceding claims, wherein the method of treating or preventing comprises administering said agent to a subject in which IL-11 or IL-11R expression has been determined to be upregulated.

23. The agent for use in a method of treating or preventing fibrosis, use or method according to any one of the preceding claims, wherein the method of treating or preventing comprises determining whether IL-11 or IL-11R expression is upregulated in the subject and administering said agent to a subject in which IL-11 or IL-11R expression is upregulated.

24. A method of determining the suitability of a subject for the treatment or prevention of fibrosis with an agent capable of inhibiting the action of Interleukin 11 (IL-11), the method comprising determining, optionally *in vitro*, whether IL-11 or an Interleukin 11 receptor (IL-11R) expression is upregulated in the subject.

25. A method of selecting a subject for the treatment or prevention of fibrosis with an agent capable of inhibiting the action of Interleukin 11 (IL-11), the method comprising

determining, optionally *in vitro*, whether IL-11 or an Interleukin 11 receptor (IL-11R) expression is upregulated in the subject.

26. A method of diagnosing fibrosis or a risk of developing fibrosis in a subject, the method comprising determining, optionally *in vitro*, the upregulation of Interleukin 11 (IL-11) or an Interleukin 11 receptor (IL-11R) in a sample obtained from the subject.

27. The method of claim 26, wherein the method is a method of confirming a diagnosis of fibrosis in a subject suspected of having fibrosis.

28. The method of claim 26 or 27, wherein the method further comprises selecting the subject for treatment with an agent capable of inhibiting the action of IL-11 or with an agent capable of preventing or reducing the expression of IL-11 or IL-11R.

29. A method of providing a prognosis for a subject having, or suspected of having fibrosis, the method comprising determining, optionally *in vitro*, whether Interleukin 11 (IL-11) or an Interleukin 11 receptor (IL-11R) is upregulated in a sample obtained from the subject and, based on the determination, providing a prognosis for treatment of the subject with an agent capable of inhibiting the action of IL-11 or with an agent capable of preventing or reducing the expression of IL-11 or IL-11R.

30. The method of claim 29, wherein the method further comprises selecting a subject determined to have upregulated IL-11 or IL-11R for treatment with an agent capable of inhibiting the action of IL-11 or with an agent capable of preventing or reducing the expression of IL-11 or IL-11R.

31. A method of diagnosing fibrosis or a risk of developing fibrosis in a subject, the method comprising determining, optionally *in vitro*, one or more genetic factors in the subject that are predictive of upregulation of Interleukin 11 (IL-11) or an Interleukin 11 receptor (IL-11R) expression, or of upregulation of IL-11 or IL-11R activity.

32. The method of claim 31, wherein the method is a method of confirming a diagnosis of fibrosis in a subject suspected of having fibrosis.

33. The method of claim 32 or 32, wherein the method further comprises selecting the subject for treatment with an agent capable of inhibiting the action of IL-11 or with an agent capable of preventing or reducing the expression of IL-11 or IL-11R.

34. A method of providing a prognosis for a subject having, or suspected of having, fibrosis, the method comprising determining, optionally *in vitro*, one or more genetic factors in the subject that are predictive of upregulation of Interleukin 11 (IL-11) or an Interleukin 11 receptor (IL-11R) expression, or of upregulation of IL-11 or IL-11R activity.

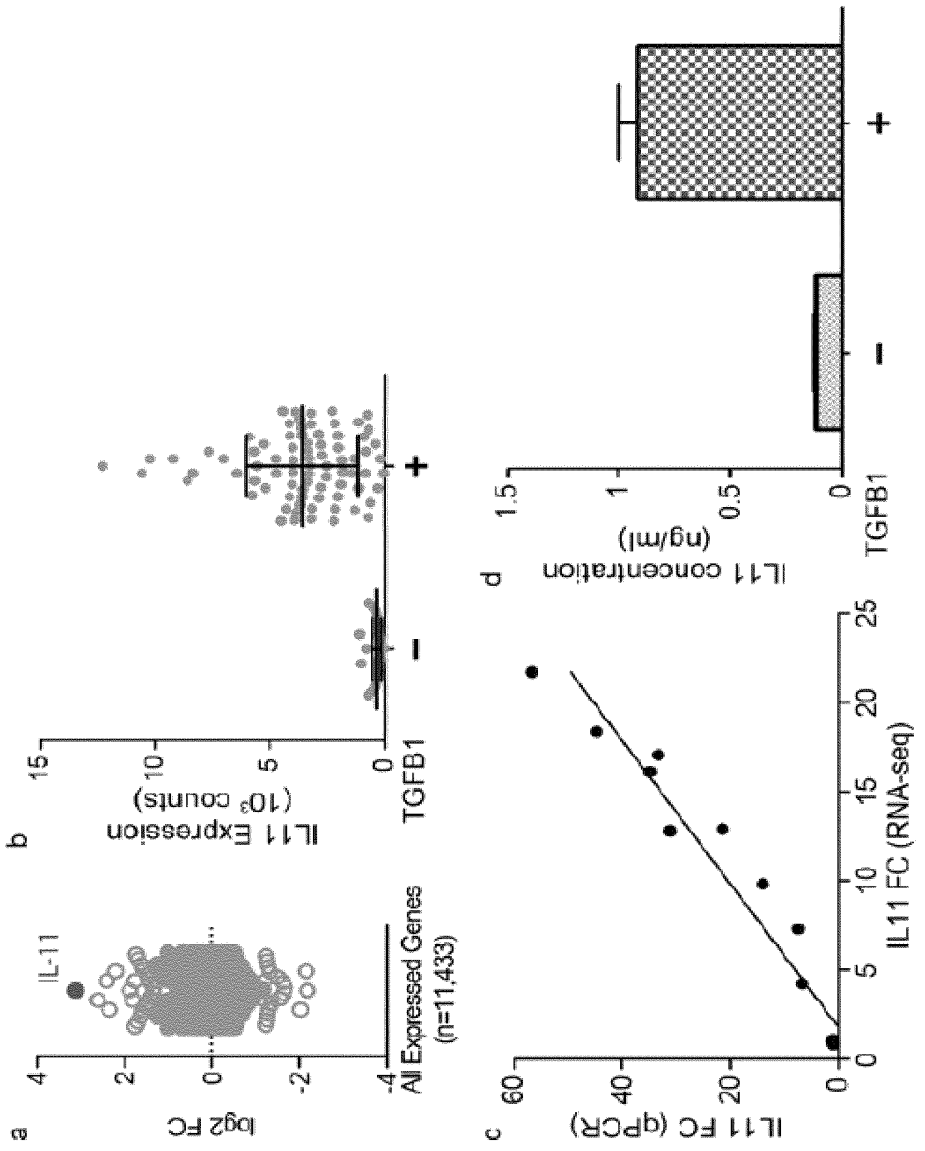

a
log2 FC
IL-11
All Expressed Genes
(n=11,433)
b
IL11 Expression
(10³ counts)
TGFB1
c
IL11 FC (qPCR)
IL11 FC (RNA-seq)
d
IL11 concentration
(ng/ml)
TGFB1

Figure 1

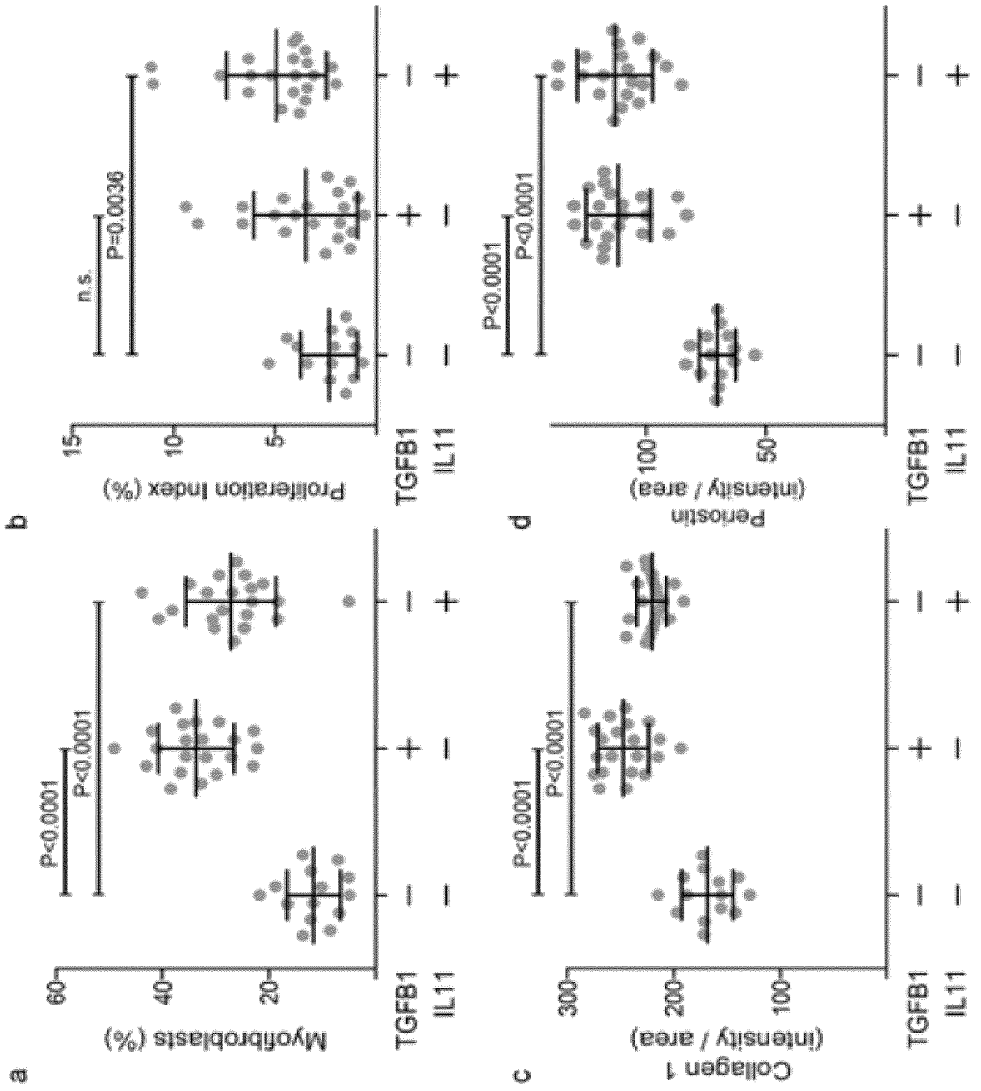

a
Myofibroblasts (%)
60
40
20
P<0.0001
P<0.0001
TGFB1 − + − +
IL11 − − + +
b
Proliferation Index (%)
15
10
5
n.s.
P=0.0036
TGFB1 − + − +
IL11 − − + +
c
Collagen 1
(intensity / area)
300
200
100
P<0.0001
P<0.0001
TGFB1 − + − +
IL11 − − + +
d
Periostin
(intensity / area)
100
50
P<0.0001
P<0.0001
TGFB1 − + − +
IL11 − − + +

Figure 2

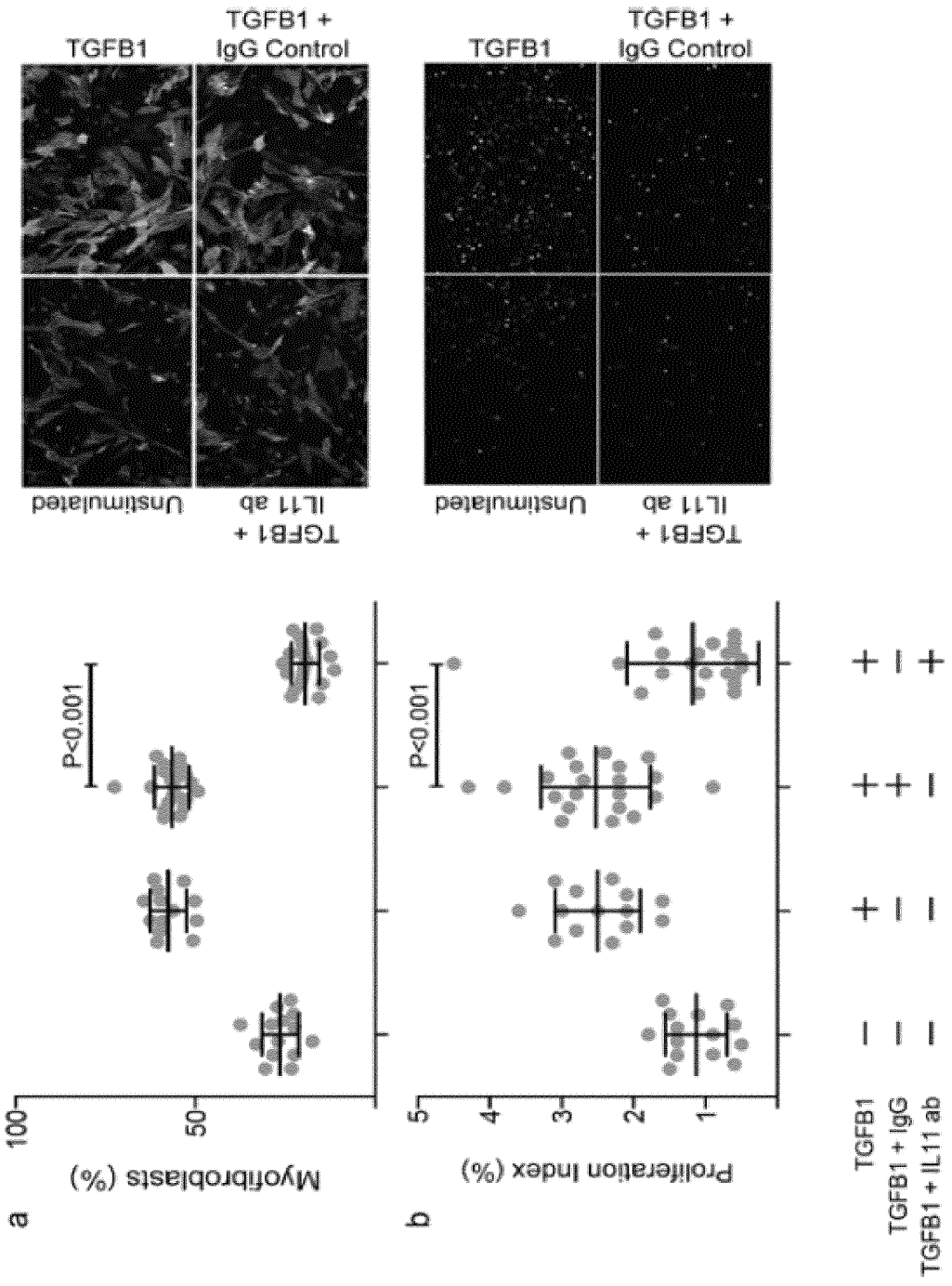


Figure 3a and 3b

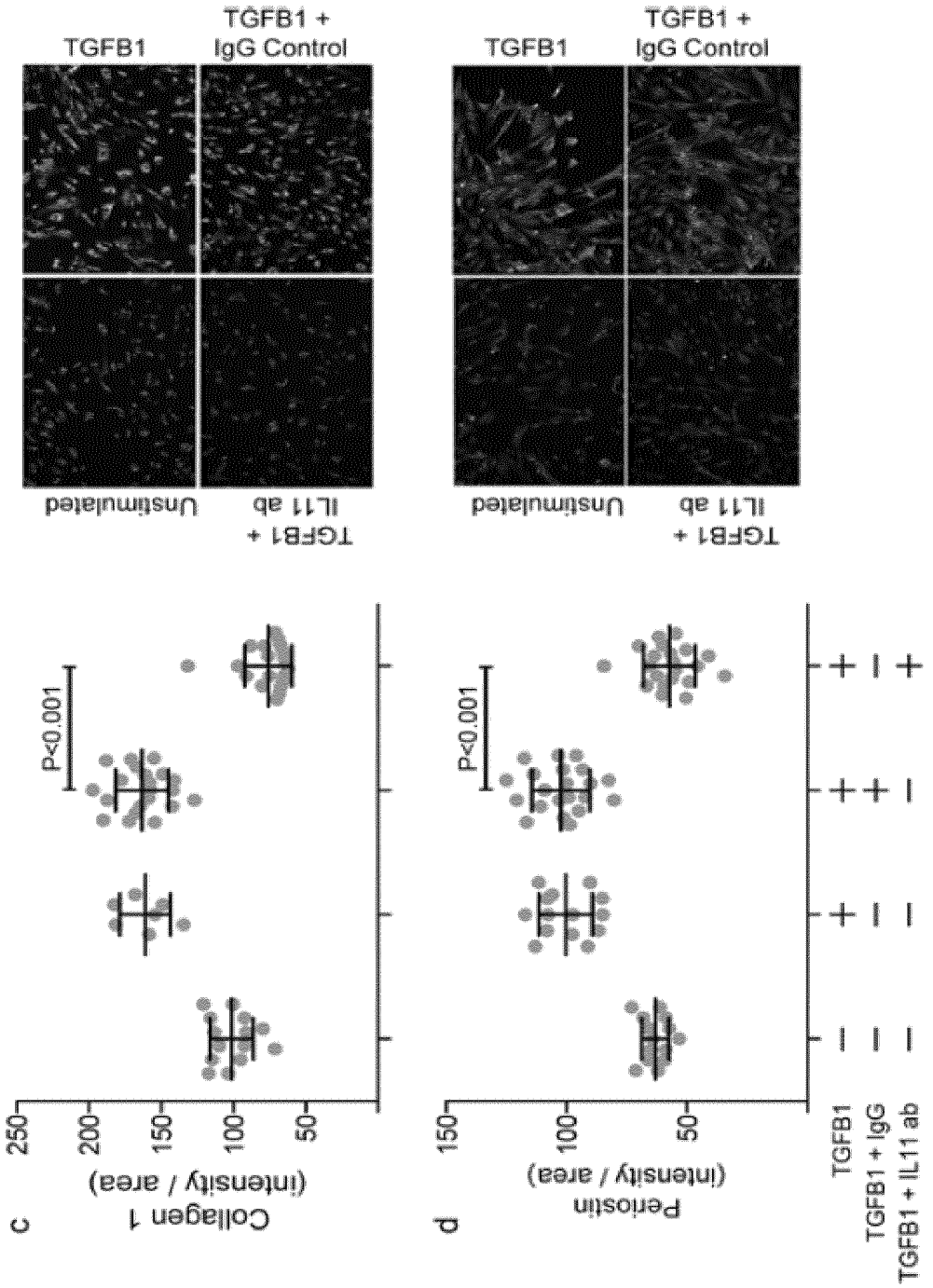
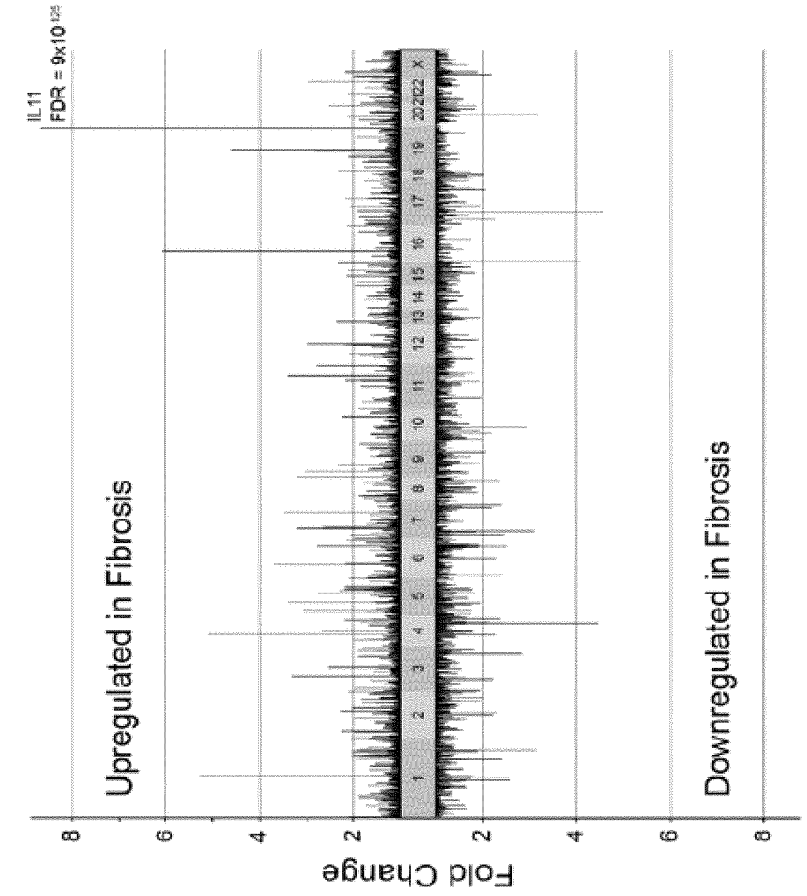


Figure 3c and 3d


IL11
FDR = 9x10
Upregulated in Fibrosis
Downregulated in Fibrosis
Fold Change
8
6
4
2
2
4
6
8
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X

a

Figure 4a

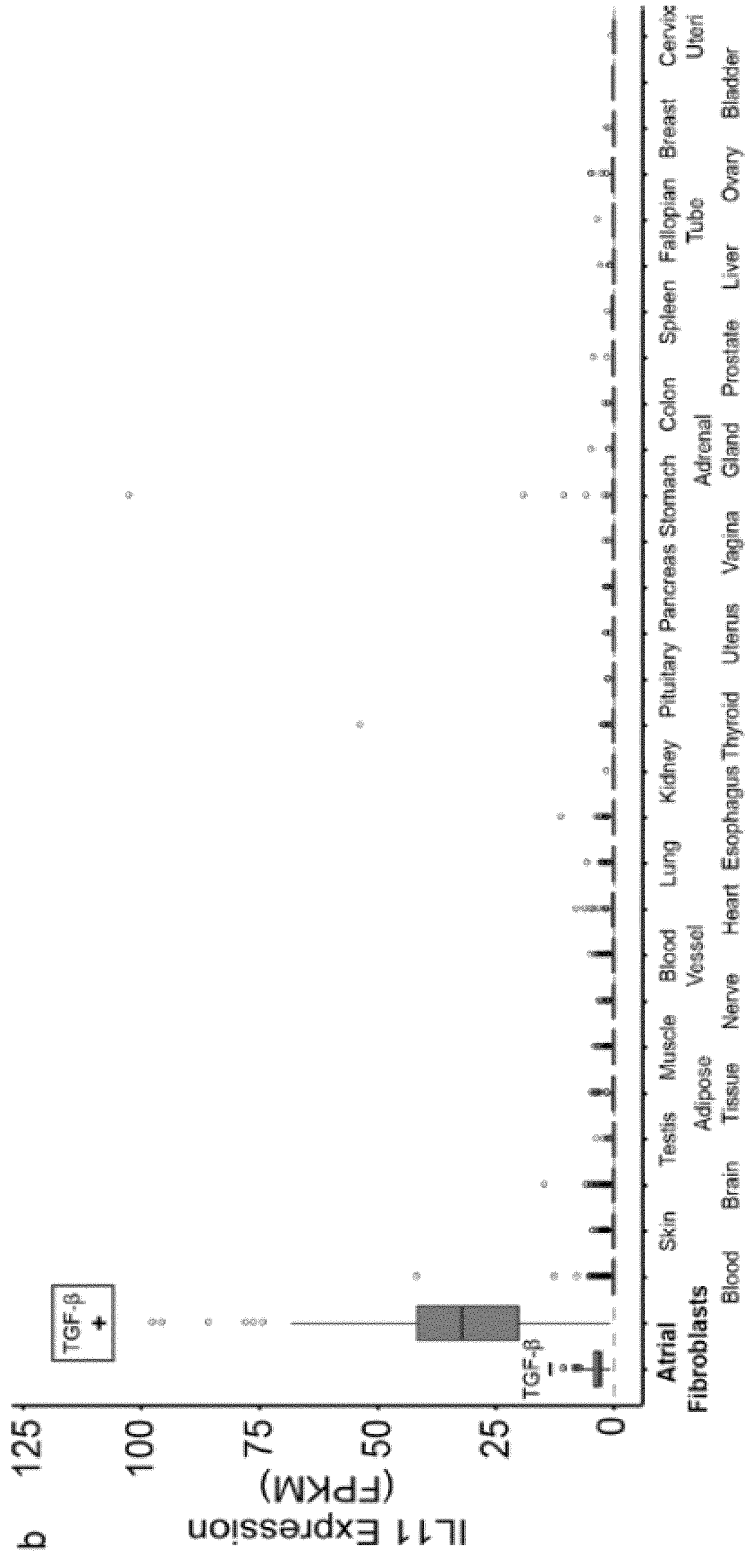

b
IL11 Expression (FPKM)
125
100
75
50
25
0
TGF-β
+
TGF-β
−
Atrial Fibroblasts
Blood
Skin
Brain
Testis
Tissue
Adipose
Muscle
Nerve
Blood Vessel
Heart
Lung
Esophagus
Kidney
Thyroid
Pituitary
Uterus
Pancreas
Vagina
Stomach
Adrenal Gland
Colon
Prostate
Spleen
Liver
Fallopian Tube
Ovary
Breast
Cervix Uteri
Bladder

Figure 4b

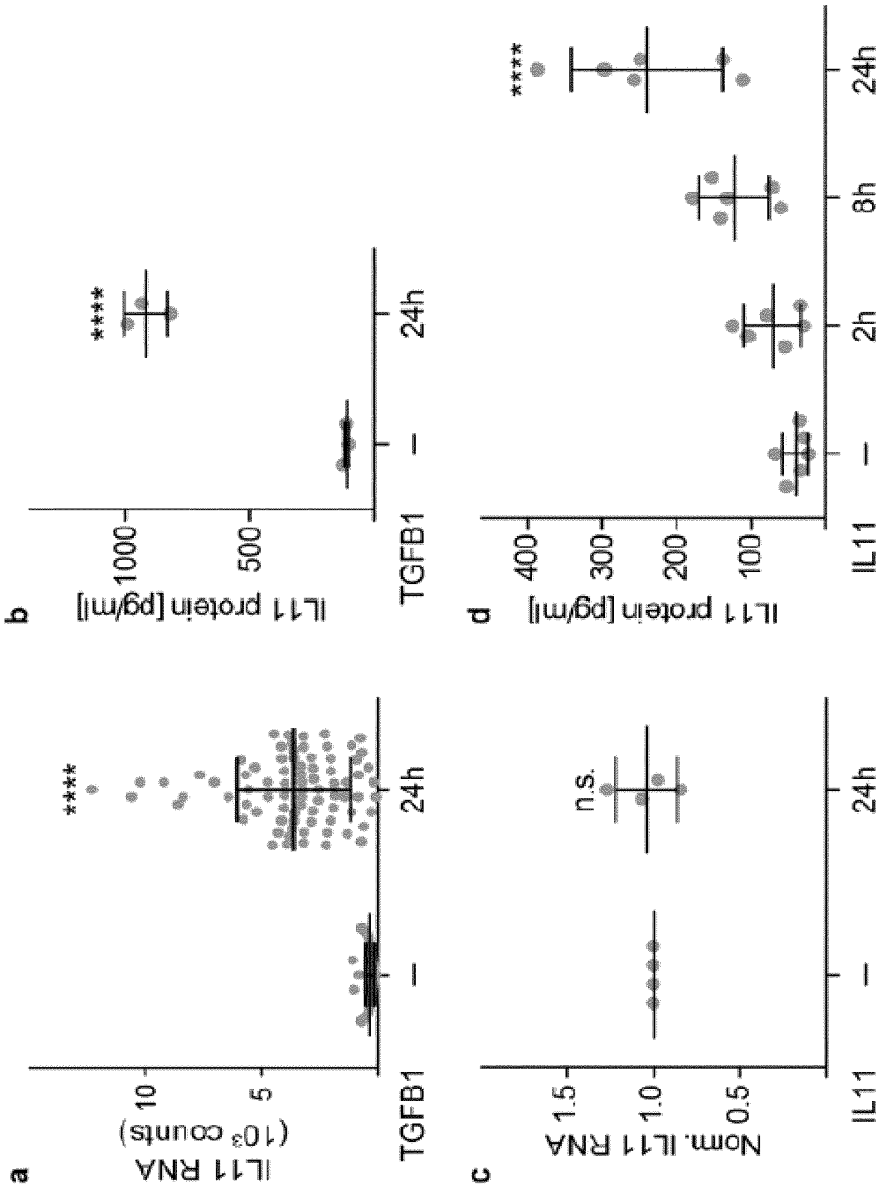

a
IL11 RNA (10³ counts)
10
5
****
—
24h
TGFB1
b
IL11 protein [pg/ml]
1000
500
****
—
24h
TGFB1
c
Nom. IL11 RNA
1.5
1.0
0.5
n.s.
—
24h
IL11
d
IL11 protein [pg/ml]
400
300
200
100
****
—
2h
8h
24h
IL11

Figure 5

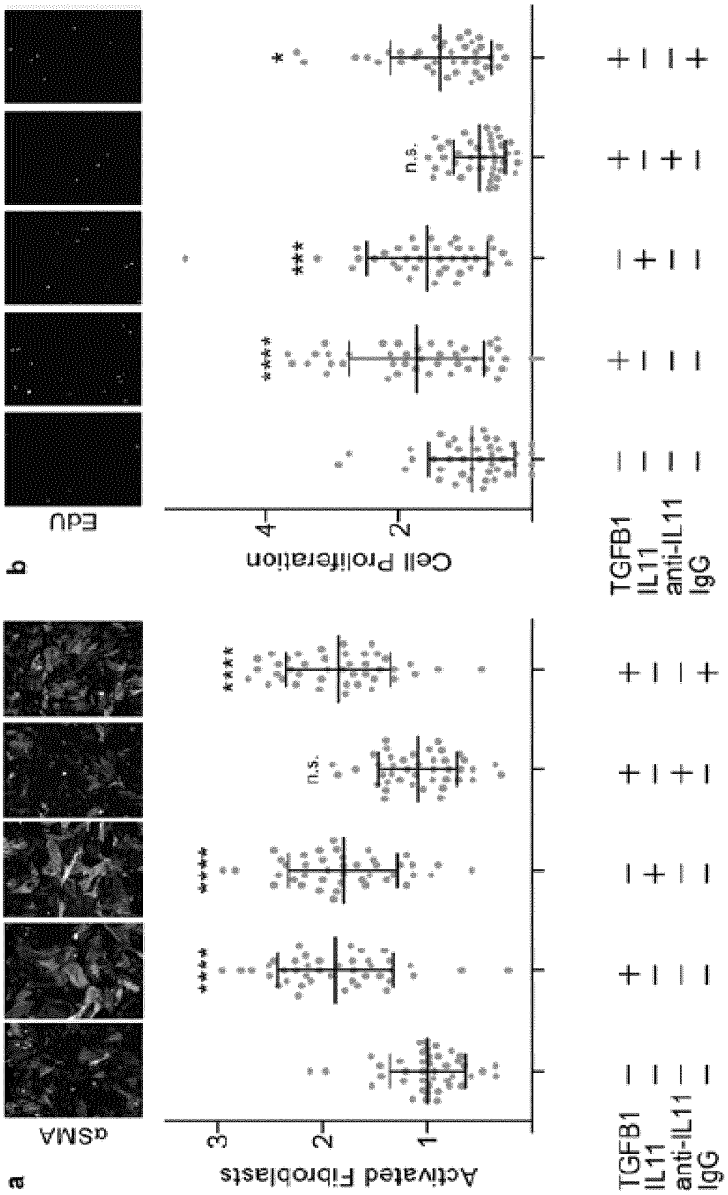


Figure 6a and 6b

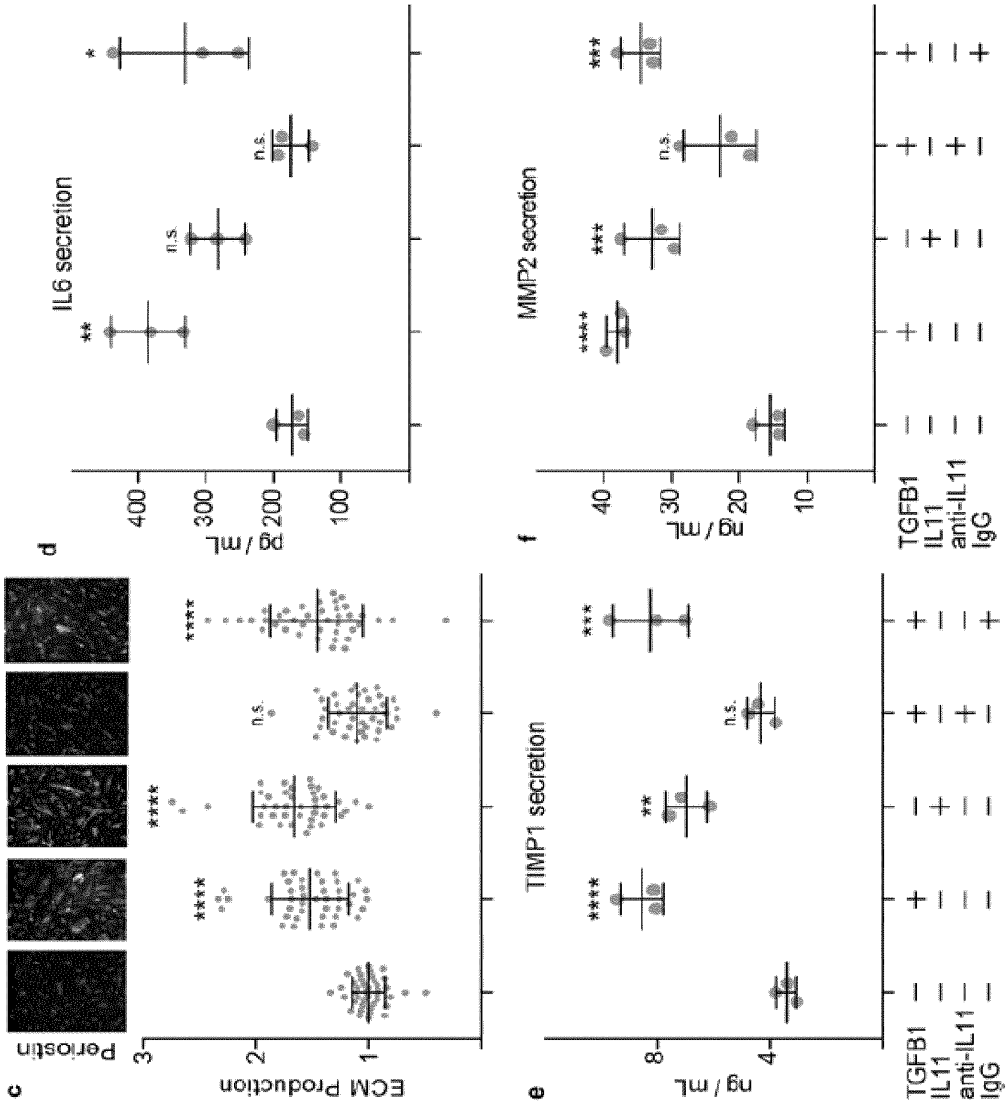


Figure 6c, 6d, 6e and 6f

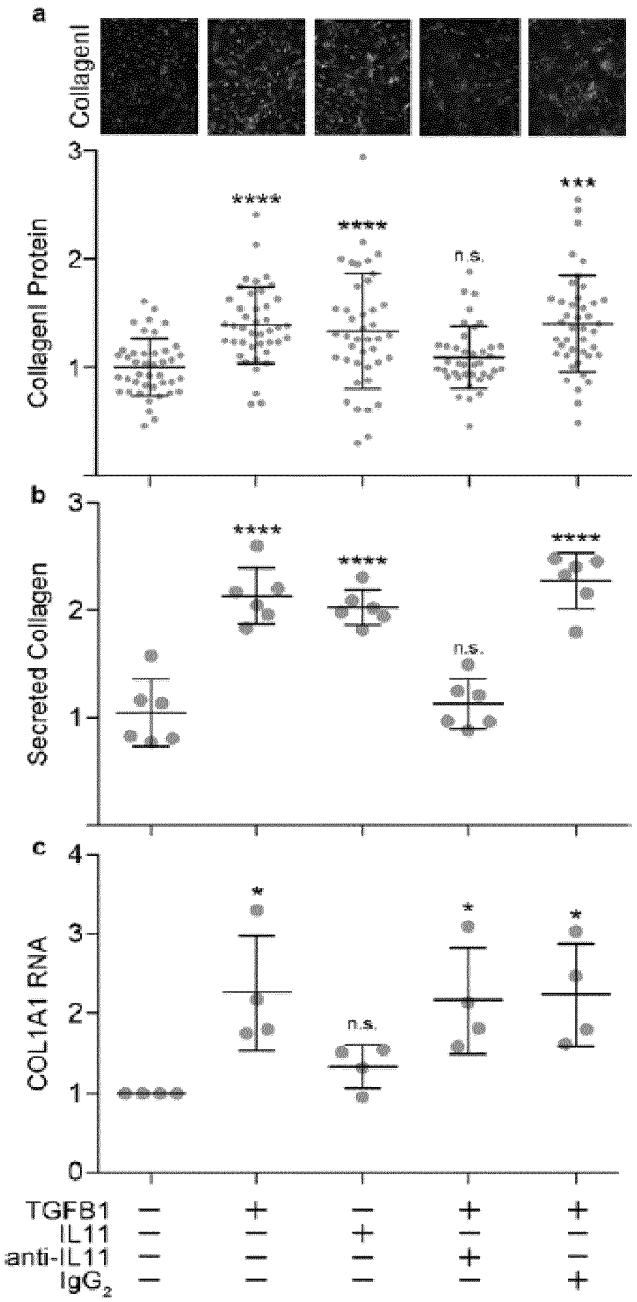

a
CollagenI
CollagenI Protein
****
****
n.s.
***
b
Secreted Collagen
****
****
n.s.
****
c
COL1A1 RNA
*
n.s.
*
*
TGFB1
IL11
anti-IL11
IgG2

Figure 7

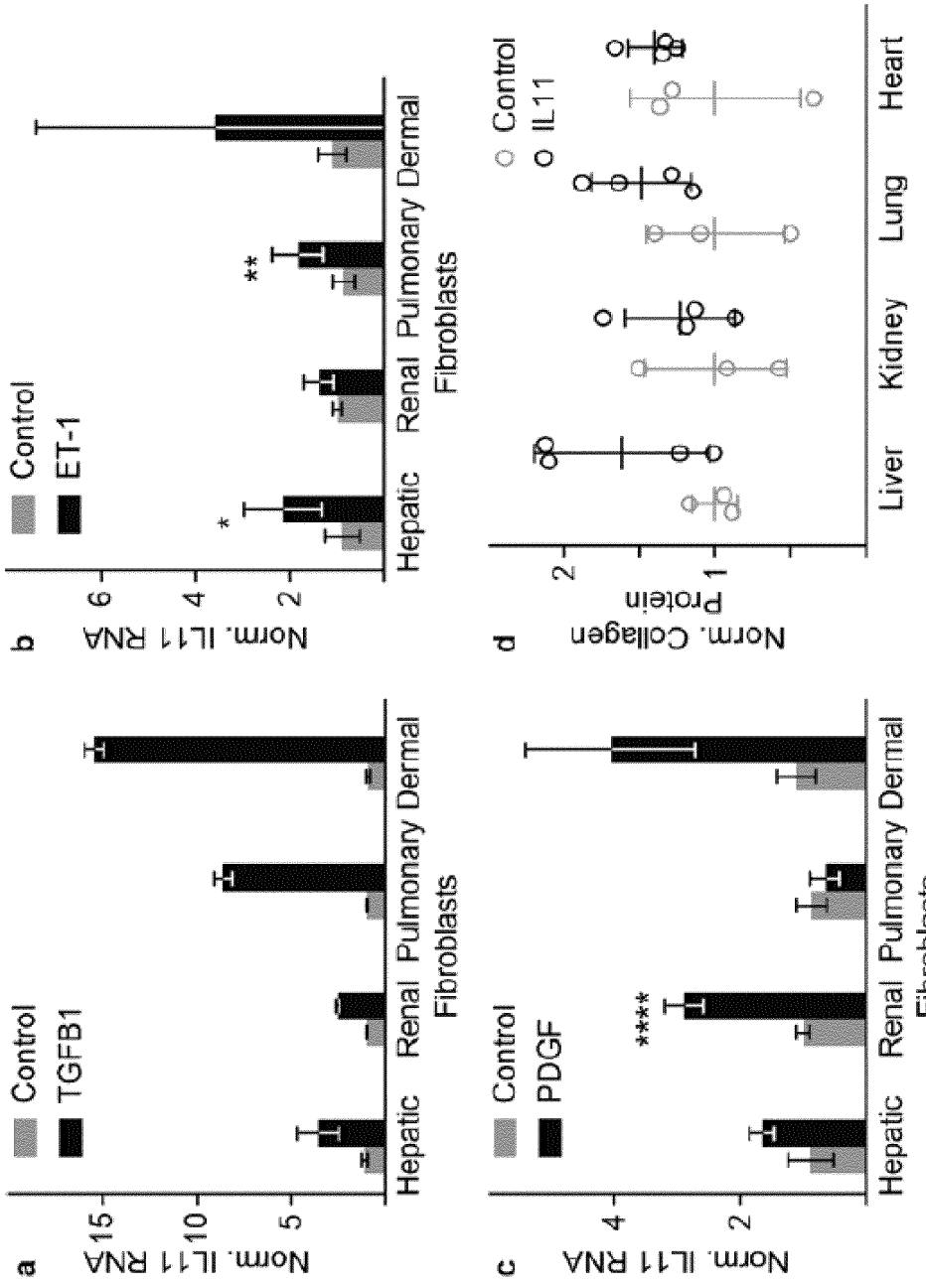


Figure 8a, 8b, 8c and 8d

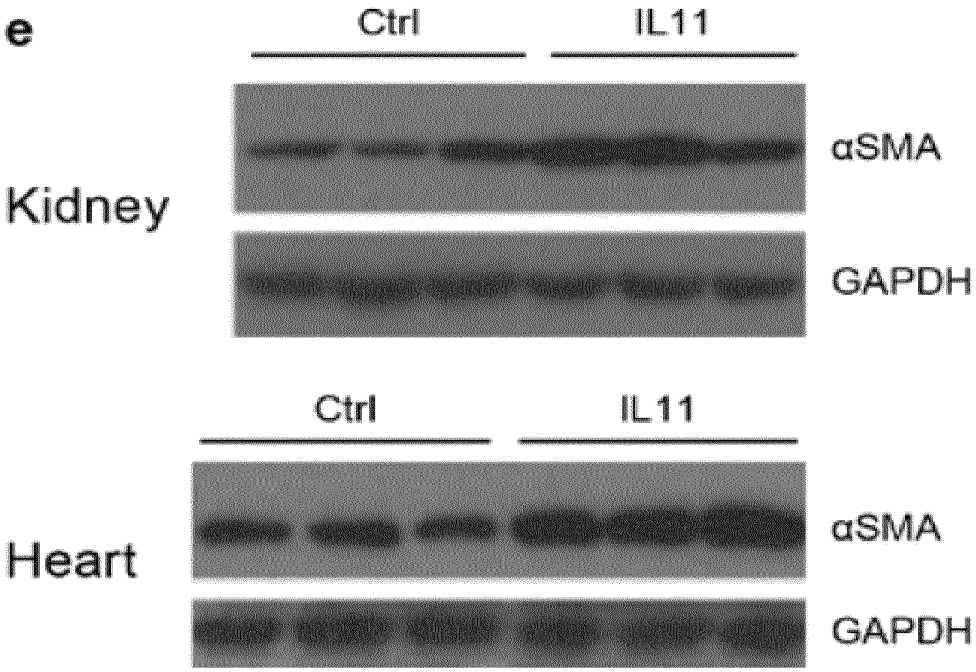


Figure 8e

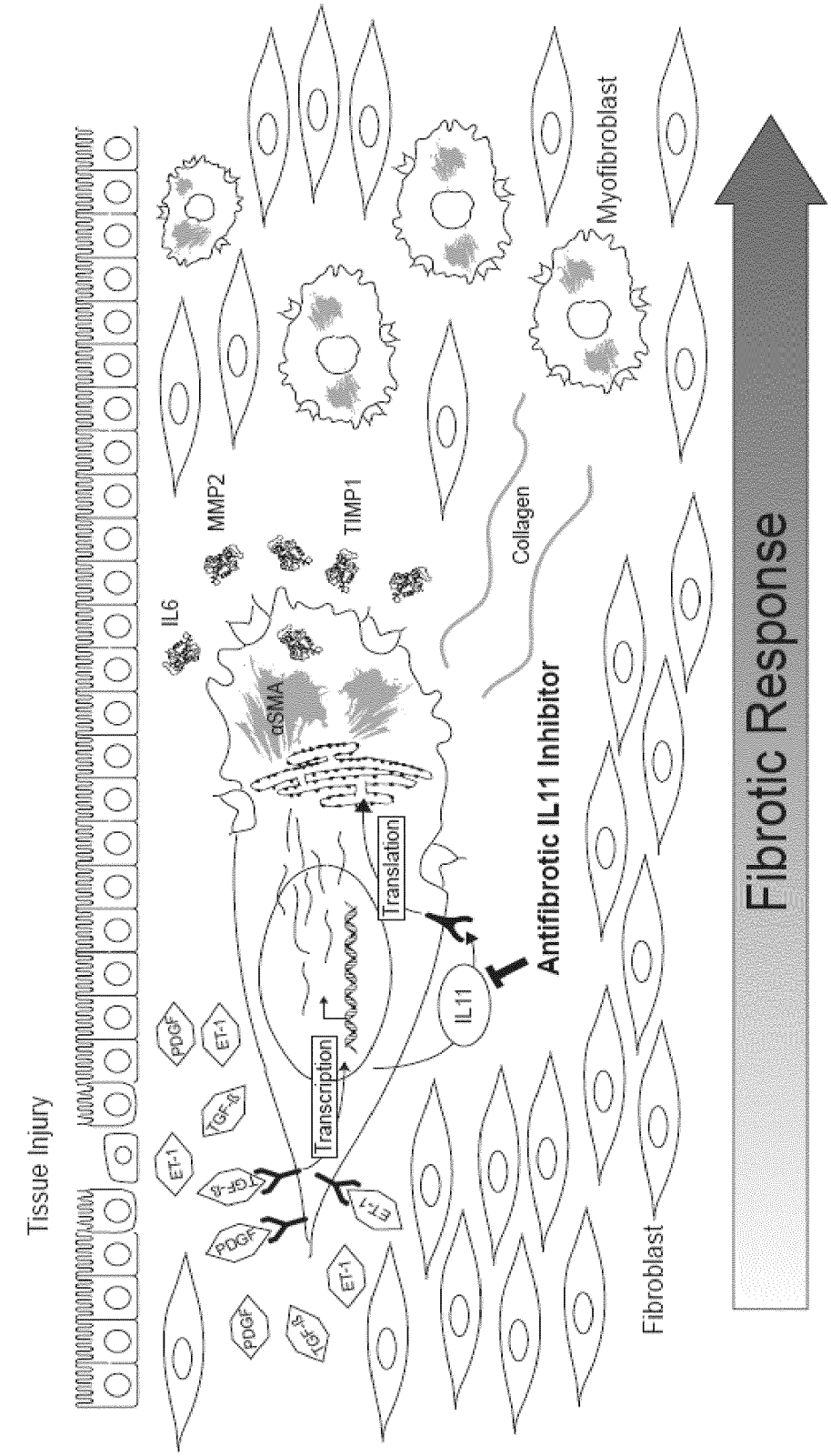

Tissue Injury
PDGF
ET-1
TGF-β
PDGF
ET-1
TGF-β
ET-1
PDGF
TGF-β
ET-1
Transcription
Translation
αSMA
IL6
MMP2
TIMP1
IL11
Antifibrotic IL11 Inhibitor
Collagen
Myofibroblast
Fibroblast
Fibrotic Response

Figure 9

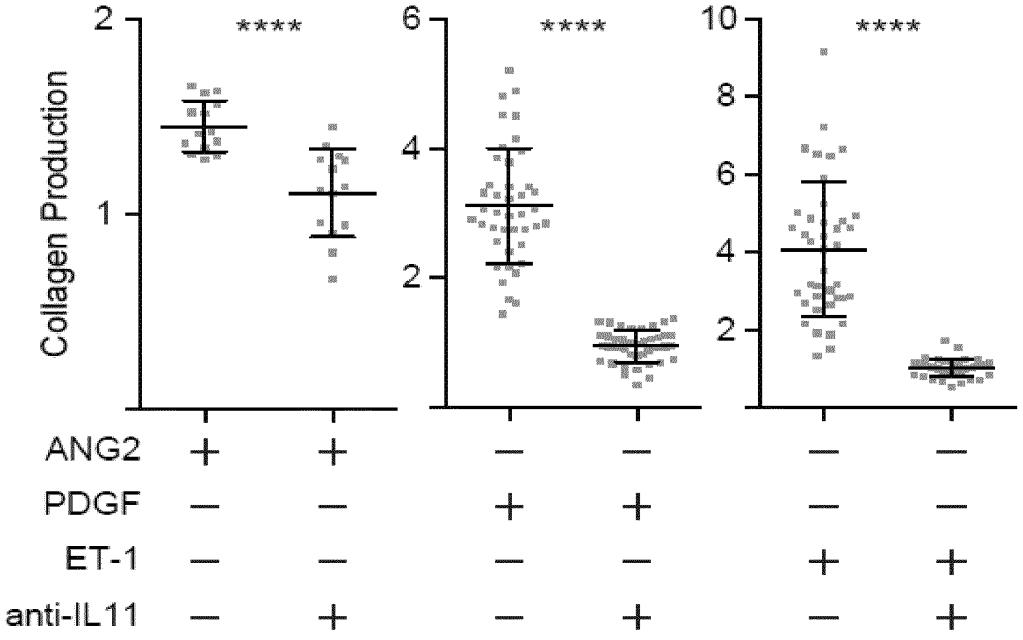


Figure 10

ACTGCCGCGGCCCTGCTGCTCAGGGCACATGCCTCCCCTCCCCAGGCCGCGGCCCAGCTGACCCTCGGGG
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GCCCTGTGGGGACATGAACTGTGTTTGCCGCCTGGTCCTGGTCGTGCTGAGCCTGTGGCCAGATACAGCT
GTCGCCCCTGGGCCACCACCTGGCCCCCCTCGAGTTTCCCCAGACCCTCGGGCCGAGCTGGACAGCACCG
TGCTCCTGACCCGCTCTCTCCTGGCGGACACGCGGCAGCTGGCTGCACAGCTGAGGGACAAATTCCCAGC
TGACGGGGACCACAACCTGGATTCCCTGCCCACCCTGGCCATGAGTGCGGGGGCACTGGGAGCTCTACAG
CTCCCAGGTGTGCTGACAAGGCTGCGAGCGGACCTACTGTCCTACCTGCGGCACGTGCAGTGGCTGCGCC
GGGCAGGTGGCTCTTCCCTGAAGACCCTGGAGCCCGAGCTGGGCACCCTGCAGGCCCGACTGGACCGGCT
GCTGCGCCGGCTGCAGCTCCTGATGTCCCGCCTGGCCCTGCCCCAGCCACCCCCGGACCCGCCGGCGCCC
CCGCTGGCGCCCCCCTCCTCAGCCTGGGGGGGGCATCAGGGCCGCCCACGCCATCCTGGGGGGGCTGCACC
TGACACTTGACTGGGCCGTGAGGGGACTGCTGCTGCTGAAGACTCGGCTGTGACCCGGGGCCCAAAGCCA
CCACCGTCCTTCCAAAGCCAGATCTTATTTATTTATTTATTTCAGTACTGGGGGCGAAACAGCCAGGTGA
TCCCCCCGCCATTATCTCCCCCTAGTTAGAGACAGTCCTTCCGTGAGGCCTGGGGGGCATCTGTGCCTTA
TTTATACTTATTTATTTCAGGAGCAGGGGTGGGAGGCAGGTGGACTCCTGGGTCCCCGAGGAGGAGGGGA
CTGGGGTCCCGGATTCTTGGGTCTCCAAGAAGTCTGTCCACAGACTTCTGCCCTGGCTCTTCCCCATCTA
GGCCTGGGCAGGAACATATATTATTTATTTAAGCAATTACTTTTCATGTTGGGGTGGGGACGGAGGGGAA
AGGGAAGCCTGGGTTTTTGTACAAAAATGTGAGAAACCTTTGTGAGACAGAGAACAGGGAATTAAATGTG
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TCACCCAGACAGCTCTGTGGAGGCAGGGTCTGAGCCTTGCCTGGGGCCCCGCACTGCATAGGGCCTTTTG
TTTGTTTTTTGAGATGGAGTCTCGCTCTGTTGCCTAGGCTGGAGTGCAGTGAGGCAATCTGAGGTCACTG
CAACCTCCACCTCCCGGGTTCAAGCAATTCTCCTGCCTCAGCCTCCCGATTAGCTGGGATCACAGGTGTG
CACCACCATGCCCAGCTAATTATTTATTTCTTTTGTATTTTTAGTAGAGACAGGGTTTCACCATGTTGGC
CAGGCTGGTTTCGAACTCCTGACCTCAGGTGATCCTCCTGCCTCGGCCTCCCAAAGTGCTGGGATTACAG
GTGTGAGCCACCACACCTGACCCATAGGTCTTCAATAAATATTTAATGGAAGGTTCCACAAGTCACCCTG
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CAAACTGGAAACAATCTAGATATCCAACAGTGAGGGTTAAGCAACATGGTGCATCTGTGGATAGAACGCC
ACCCAGCCGCCCGGAGCAGGGACTGTCATTCAGGGAGGCTAAGGAGAGAGGCTTGCTTGGGATATAGAAA
GATATCCTGACATTGGCCAGGCATGGTGGCTCACGCCTGTAATCCTGGCACTTTGGGAGGACGAAGCGAG
TGGATCACTGAAGTCCAAGAGTTCGAGACCGGCCTGCGAGACATGGCAAAACCCTGTCTCAAAAAAGAAA
GAATGATGTCCTGACATGAAACAGCAGGCTACAAAACCACTGCATGCTGTGATCCCAATTTTGTGTTTTT
CTTTCTATATATGGATTAAAACAAAAATCCTAAAGGGAAATACGCCAAAATGTTGACAATGACTGTCTCC
AGGTCAAAGGAGAGAGGTGGGATTGTGGGTGACTTTTAATGTGTATGATTGTCTGTATTTTACAGAATTT
CTGCCATGACTGTGTATTTTGCATGACACATTTTAAAAATAATAAACACTATTTTTAGAATAACAGAAAA
A [SEQ ID NO:1]

CCTTCCAAAGCCAGATCTT [SEQ ID NO:2]

GCCTGGGCAGGAACATATA [SEQ ID NO:3]

CCTGGGCAGGAACATATAT [SEQ ID NO:4]

GGTTCATTATGGCTGTGTT [SEQ ID NO:5]

Figure 11

GCTGTAGCTGGTGAGAGGAAGTCCTAGAGGCTATGGACACTCTGCTGCTGGGATCACCGAGATGAGCAGC
AGCTGCTCAGGGCTGAGCAGGGTCCTGGTGGCCGTGGCTACAGCCCTGGTGTCTGCCTCCTCCCCCTGCC
CCCAGGCCTGGGGCCCCCCAGGGGTCCAGTATGGGCAGCCAGGGAGGTCCGTGAAGCTGTGTTGTCCTGG
AGTGACTGCCGGGGACCCAGTGTCCTGGTTTCGGGATGGGGAGCCAAAGCTGCTCCAGGGACCTGACTCT
GGGCTAGGGCATGAACTGGTCCTGGCCCAGGCAGACAGCACTGATGAGGGCACCTACATCTGCCAGACCC
TGGATGGTGCACTTGGGGGCACAGTGACCCTGCAGCTGGGCTACCCTCCAGCCCGCCCTGTTGTCTCCTG
CCAAGCAGCCGACTATGAGAACTTCTCTTGCACTTGGAGTCCCAGCCAGATCAGCGGTTTACCCACCCGC
TACCTCACCTCCTACAGGAAGAAGACAGTCCTAGGAGCTGATAGCCAGAGGAGGAGTCCATCCACAGGGC
CCTGGCCATGCCCACAGGATCCCCTAGGGGCTGCCCGCTGTGTTGTCCACGGGGCTGAGTTCTGGAGCCA
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AGCATCTTGCGCCCTGACCCACCCCAGGGCCTGCGGGTAGAGTCAGTACCAGGTTACCCCCGACGCCTGC
GAGCCAGCTGGACATACCCTGCCTCCTGGCCGTGCCAGCCCCACTTCCTGCTCAAGTTCCGTTTGCAGTA
CCGTCCGGCGCAGCATCCAGCCTGGTCCACGGTGGAGCCAGCTGGACTGGAGGAGGTGATCACAGATGCT
GTGGCTGGGCTGCCCCATGCTGTACGAGTCAGTGCCCGGGACTTTCTAGATGCTGGCACCTGGAGCACCT
GGAGCCCGGAGGCCTGGGGAACTCCGAGCACTGGGACCATACCAAAGGAGATACCAGCATGGGGCCAGCT
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CTGCATGCATGTATGTAGGTGCCTGGGAGTGTGTGTGGTCCTTGCTCTGGCCCTTTCCCTTGCAGGGTTG
TGCAGGTGTGAATAAA [SEQ ID NO:6]

GGACCATACCAAAGGAGAT [SEQ ID NO:7]

GCGTCTTTGGGAATCCTTT [SEQ ID NO:8]

GCAGGACAGTAGATCCCT [SEQ ID NO:9]

GCTCAAGGAACGTGTGTAA [SEQ ID NO:10]

Figure 12

Gene Name	*Gene ID/ Accession no.*	*siRNA name*	*siRNA sequence(5'-3')*	*SEQ ID NO:*
IL-11	NM_000641.3	siRNA 1	CCUUCCAAAGCCAGAUCUUdTdT AAGAUCUGGCUUUGGAAGGdTdT	11
IL-11	NM_000641.3	siRNA 2	GCCUGGGCAGGAACAUAUAdTdT UAUAUGUUCCUGCCCAGGCdTdT	12
IL-11	NM_000641.3	siRNA 3	CCUGGGCAGGAACAUAUAUdTdT AUAUAUGUUCCUGCCCAGGdTdT	13
IL-11	NM_000641.3	siRNA 4	GGUUCAUUAUGGCUGUGUGUUdTdT AACACAGCCAUAAUGAACCdTdT	14

Figure 13

Gene Name	*Gene ID/ Accession no.*	*siRNA name*	*siRNA sequence(5'-3')*	*SEQ ID NO:*
IL-11R	U32324.1	siRNA 5	GGACCAUACCAAAGGAGAUdTdT AUCUCCUUUGGUAUGGUCCdTdT	15
IL-11R	U32324.1	siRNA 6	GCGUCUUUGGGAAUCCUUUdTdT AAAGGAUUCCCAAAGACGCdTdT	16
IL-11R	U32324.1	siRNA 7	GCAGGACAGUAGAUCCCUAdTdT UAGGGAUCUACUGUCCUGCdTdT	17
IL-11R	U32324.1	siRNA 8	GCUCAAGGAACGUGUGUAAdTdT UUACACACGUUCCUUGAGCdTdT	18

Figure 14

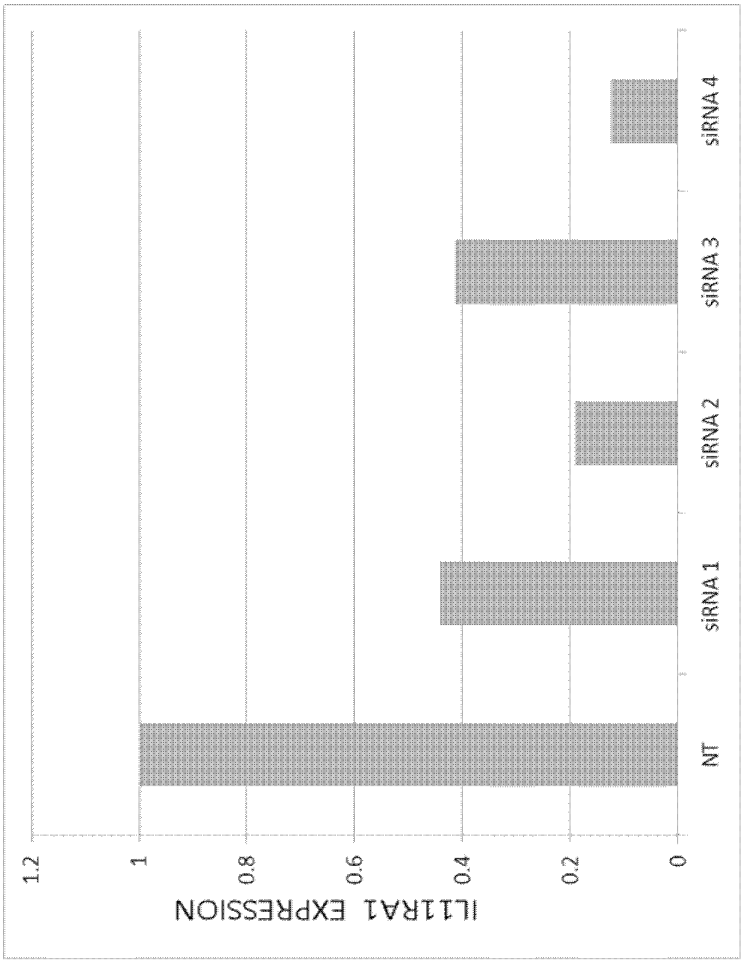
IL11RA1 EXPRESSION
1.2
1
0.8
0.6
0.4
0.2
0
NT
siRNA 1
siRNA 2
siRNA 3
siRNA 4

Figure 15

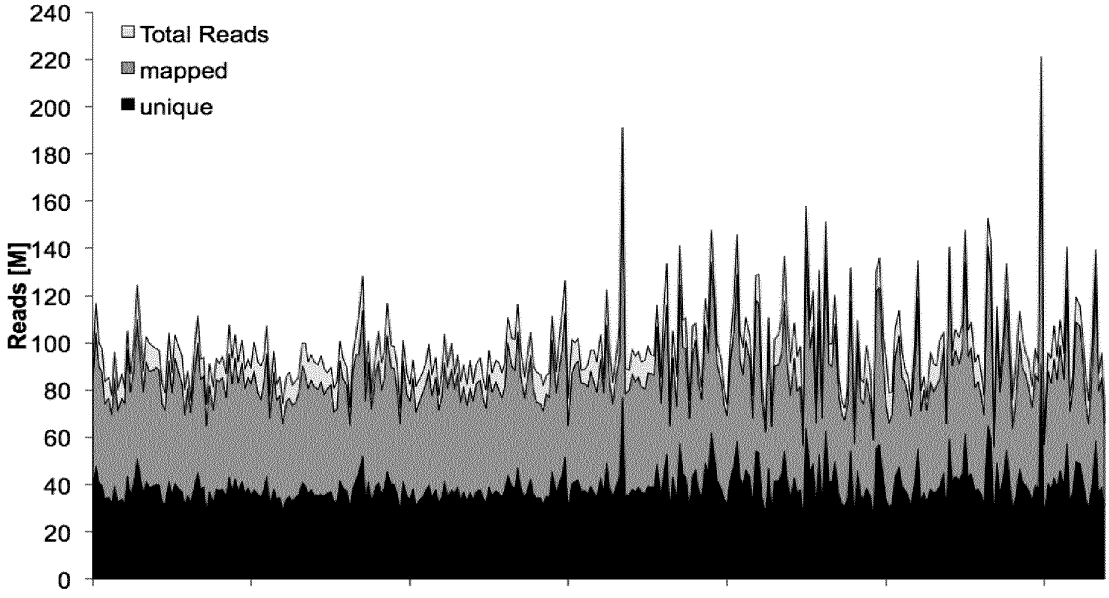

Figure 16

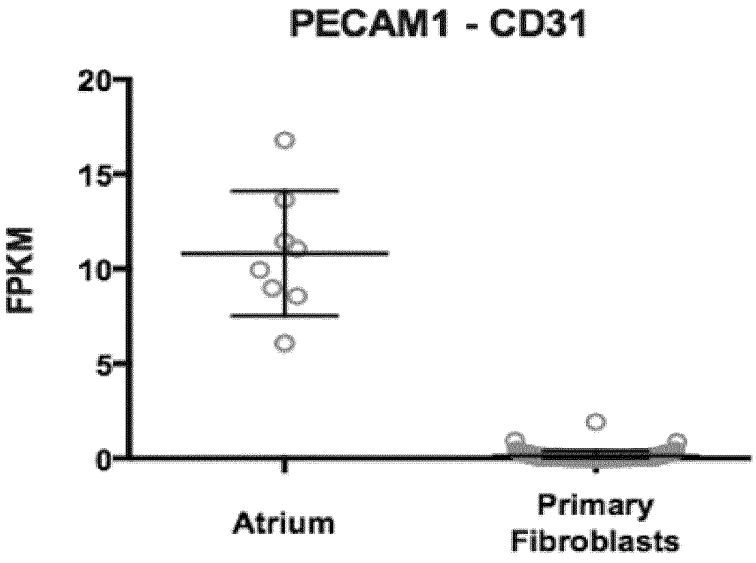

Figure 17A

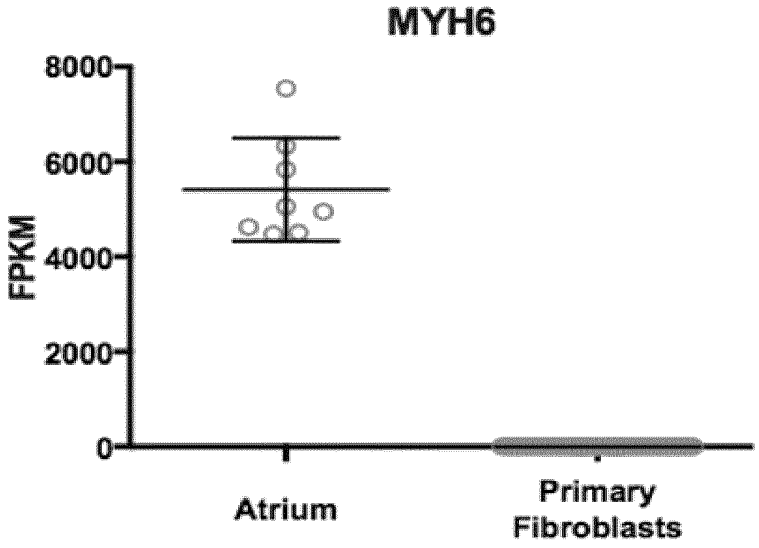

Figure 17B

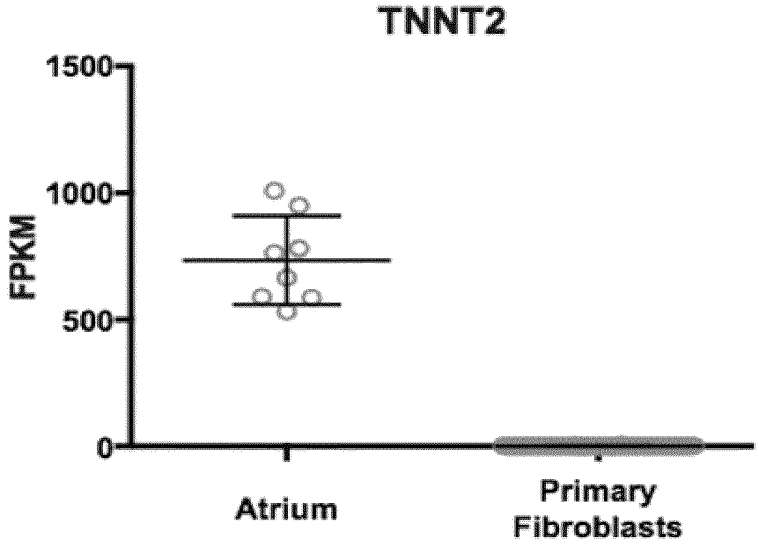

Figure 17C

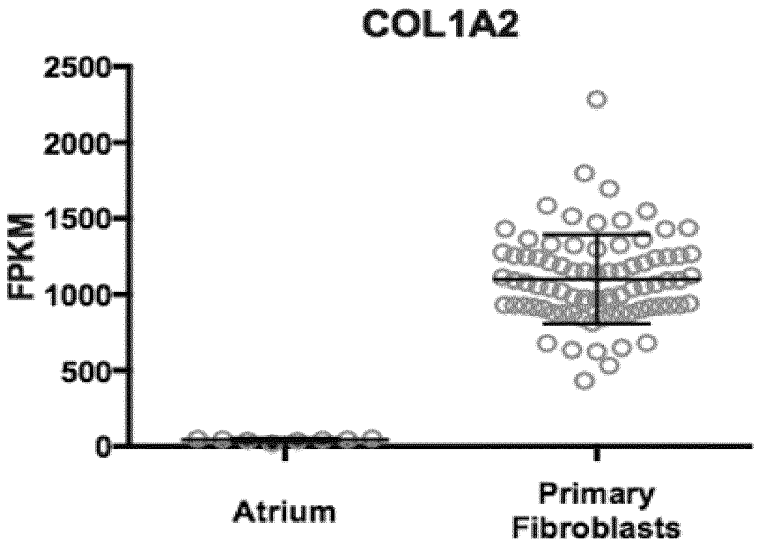

Figure 17D

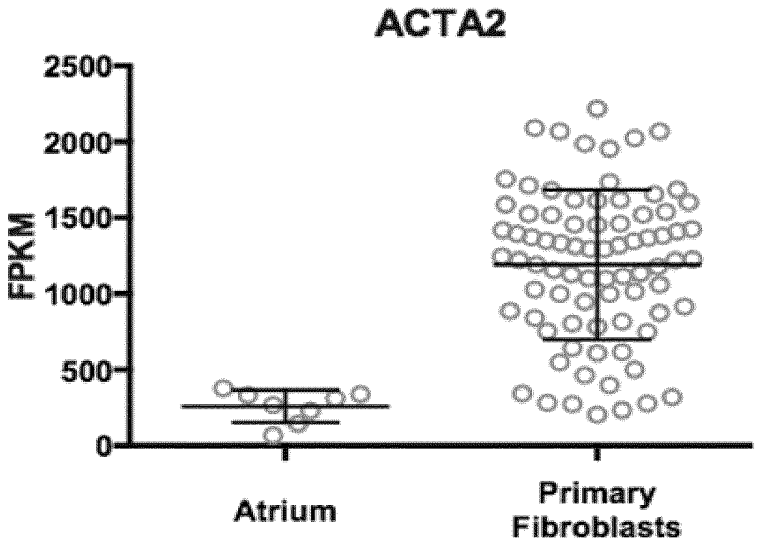

Figure 17E

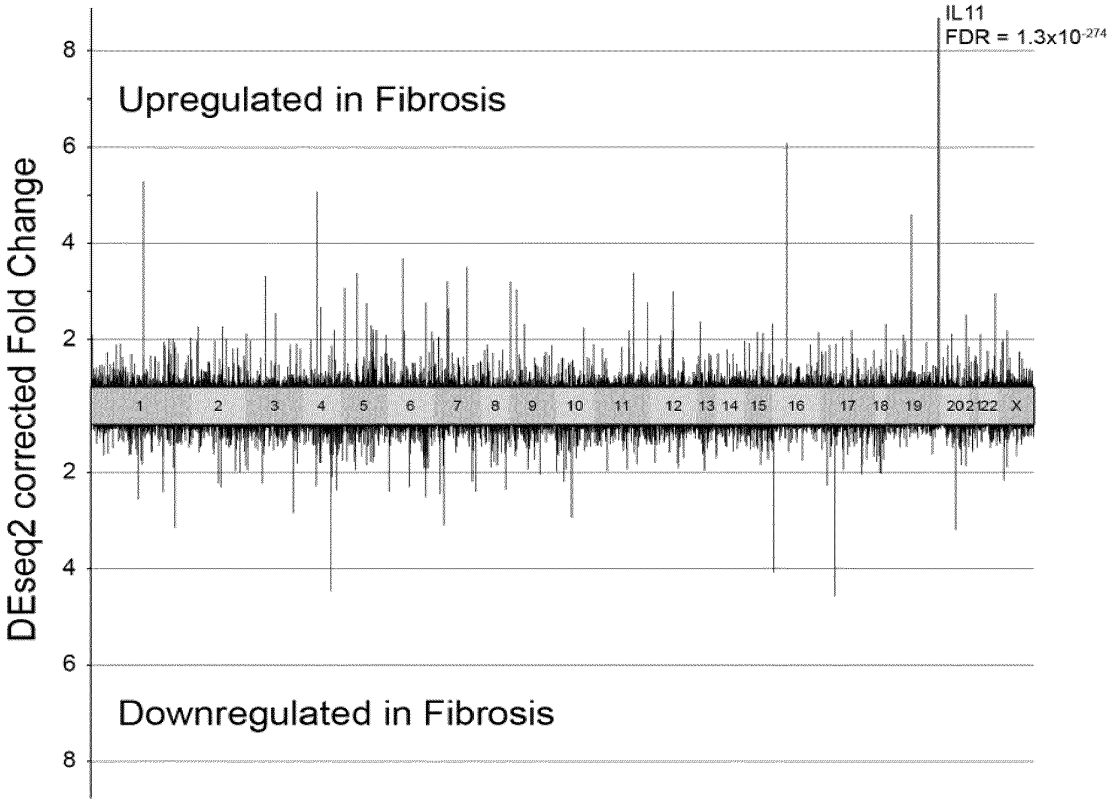

Figure 18A

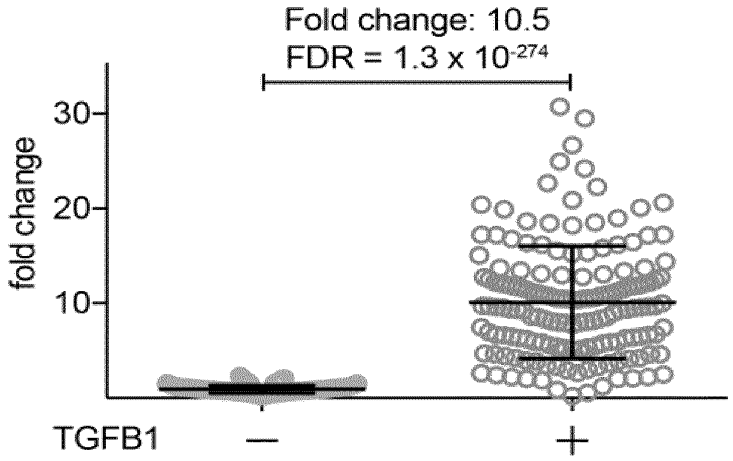

Figure 18B

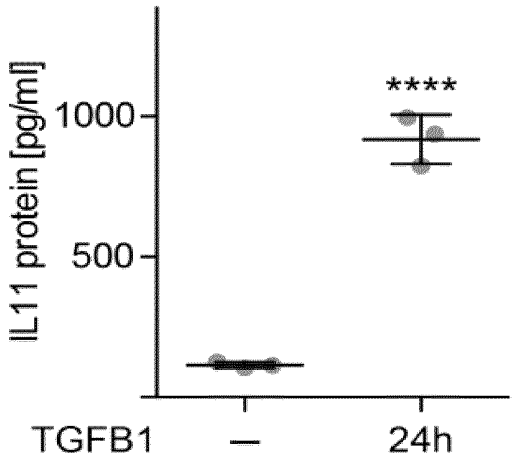

Figure 18C

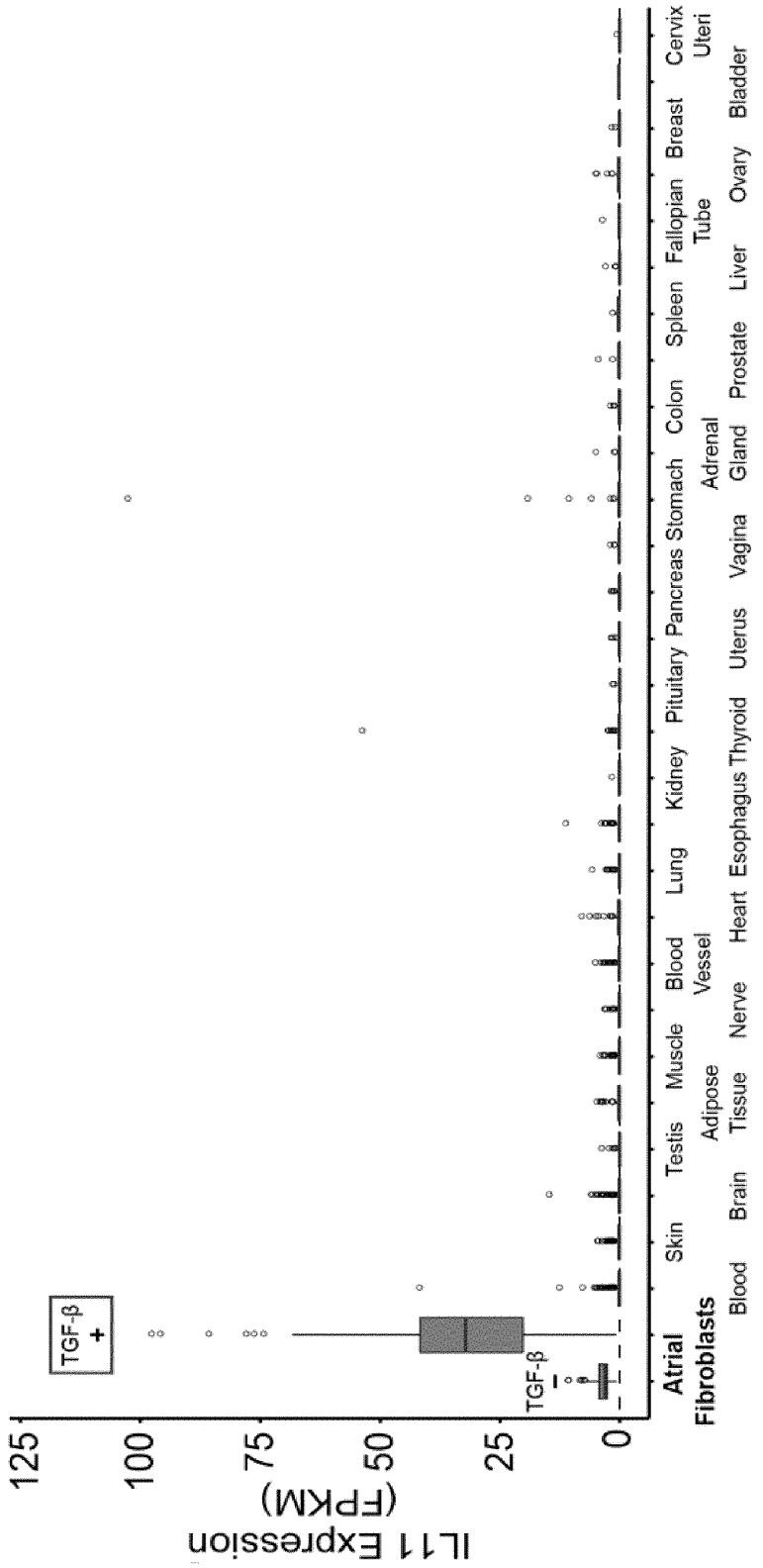
IL11 Expression (FPKM)
125
100
75
50
25
0
TGF-β +
TGF-β -
Atrial Fibroblasts
Blood
Skin
Brain
Testis
Tissue
Muscle
Adipose
Nerve
Blood Vessel
Heart
Lung
Esophagus
Kidney
Thyroid
Pituitary
Uterus
Pancreas
Vagina
Stomach
Adrenal Gland
Colon
Prostate
Spleen
Liver
Fallopian Tube
Ovary
Breast
Cervix Uteri
Bladder

Figure 18D

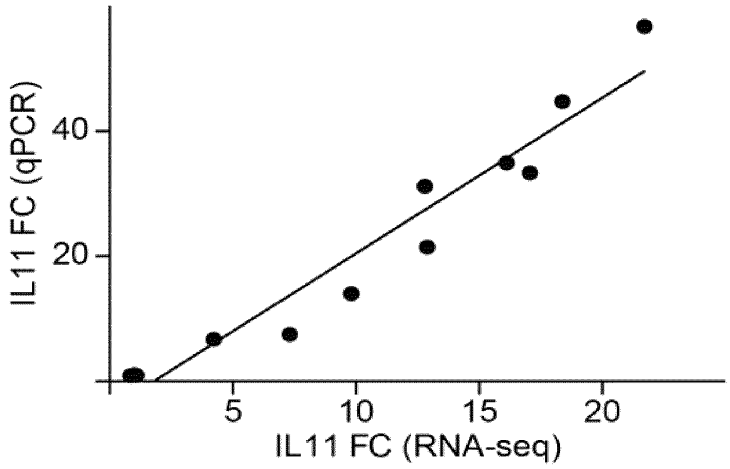

Figure 18E

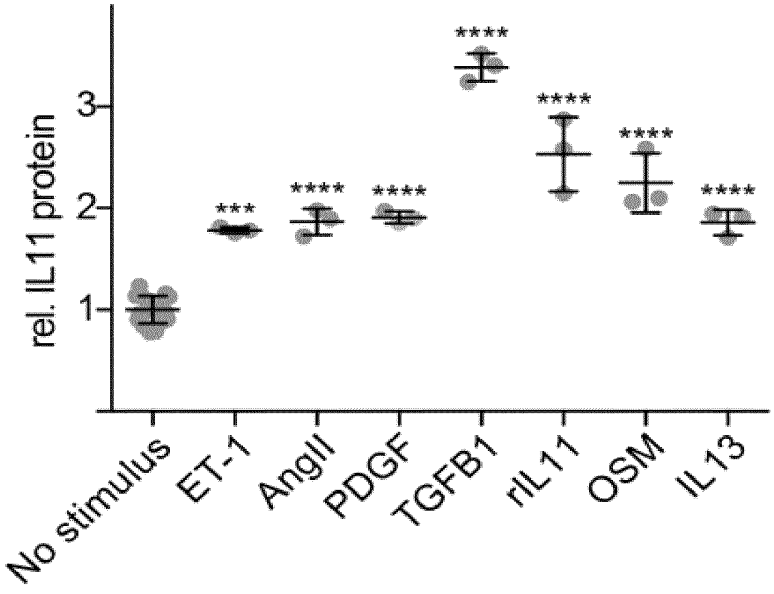

Figure 19A

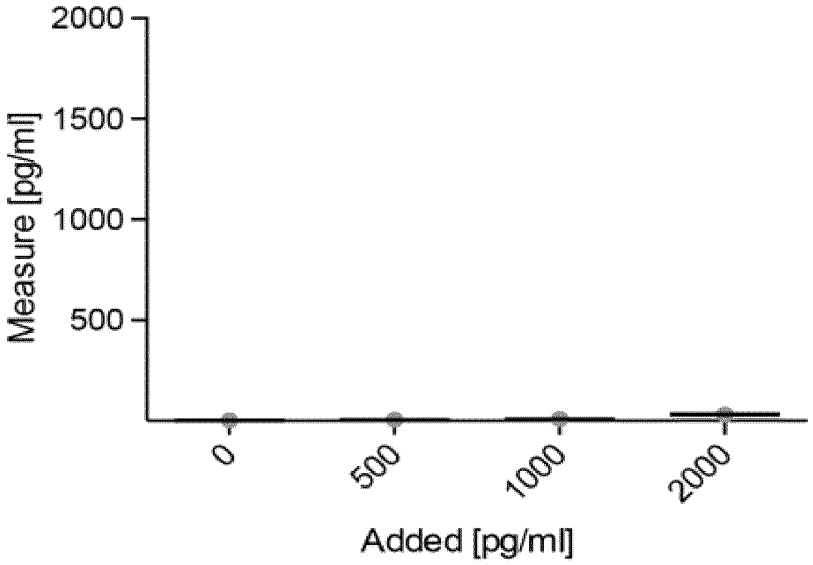

Figure 19B

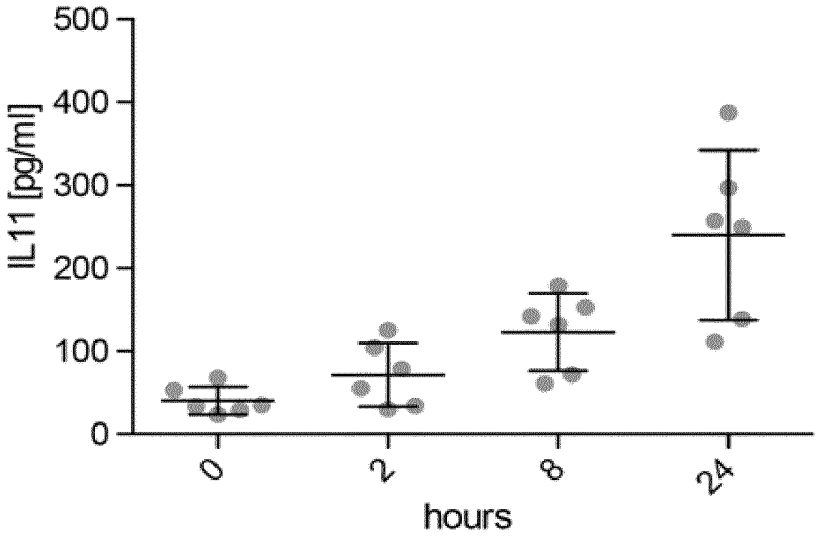

Figure 19C

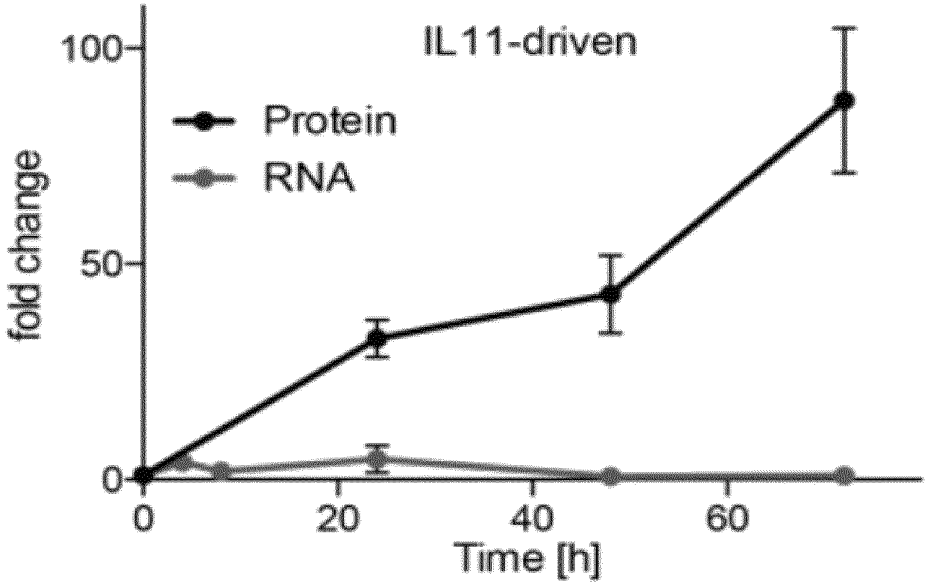

Figure 19D

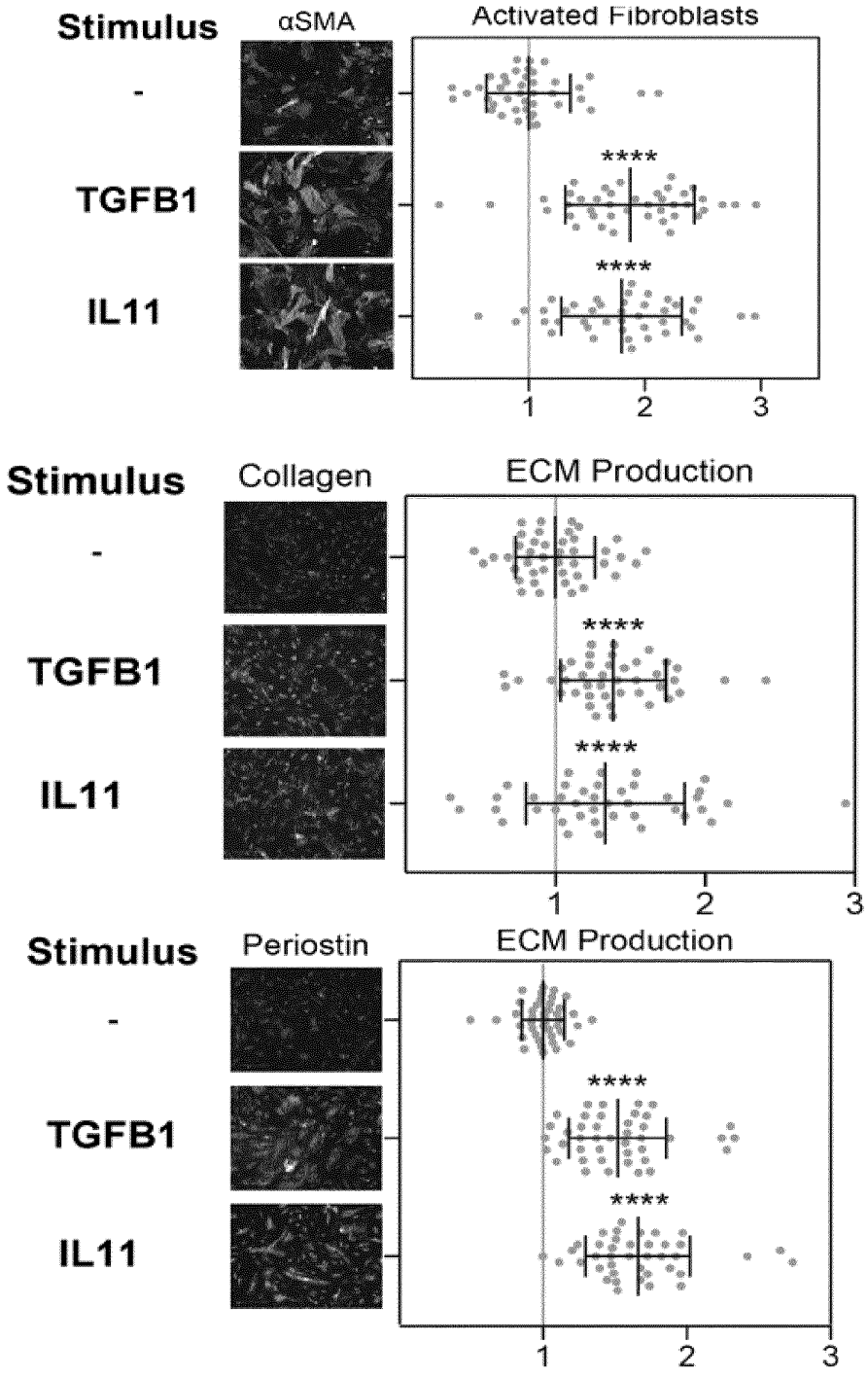
Stimulus
αSMA
Activated Fibroblasts
-
TGFB1
IL11
****
****
1
2
3
Stimulus
Collagen
ECM Production
-
TGFB1
IL11
****
****
1
2
3
Stimulus
Periostin
ECM Production
-
TGFB1
IL11
****
****
1
2
3

Figure 20A

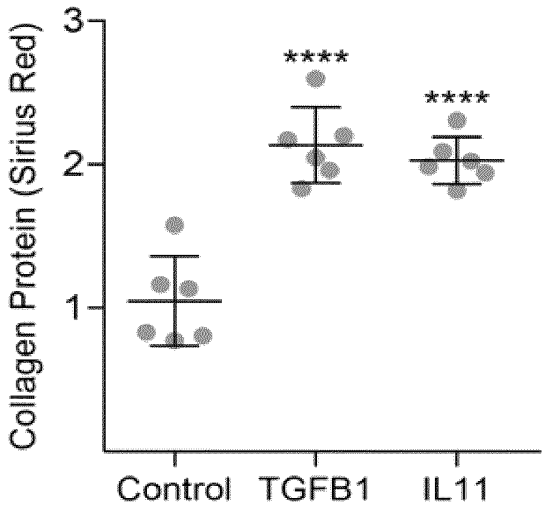

Figure 20B

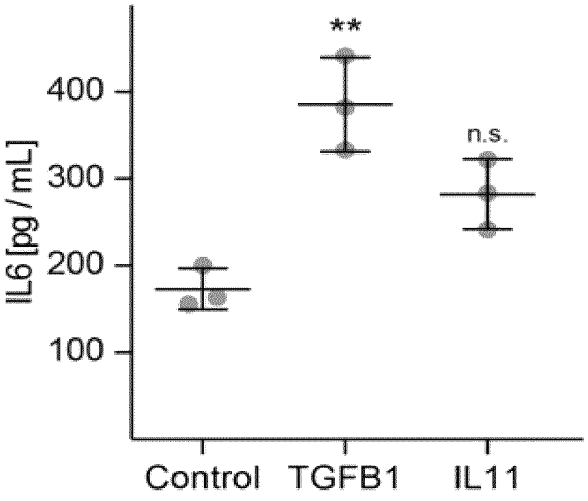

Figure 20C

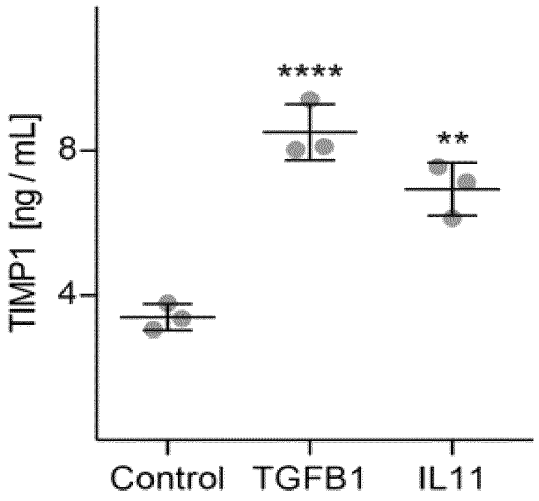

Figure 20D

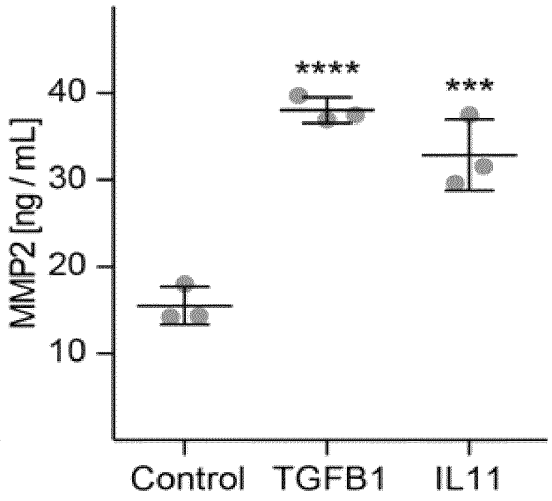

Figure 20E

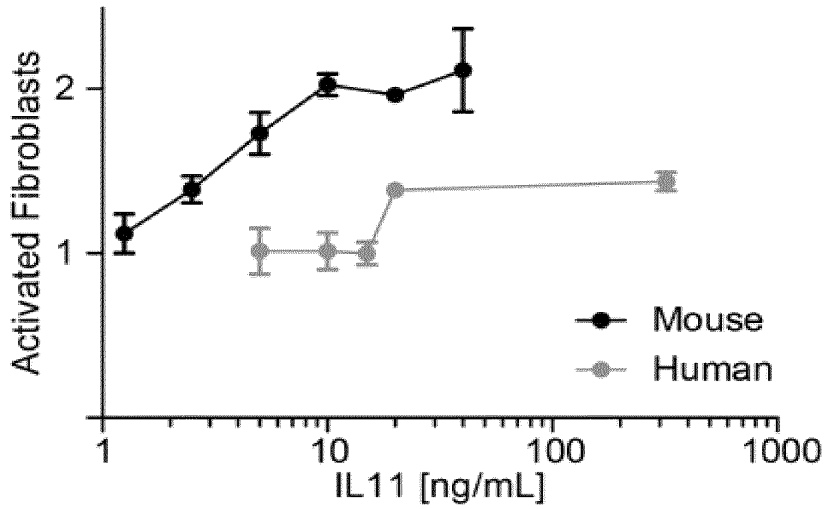
Activated Fibroblasts
2
1
1
10
100
1000
IL11 [ng/mL]
Mouse
Human

Figure 20F

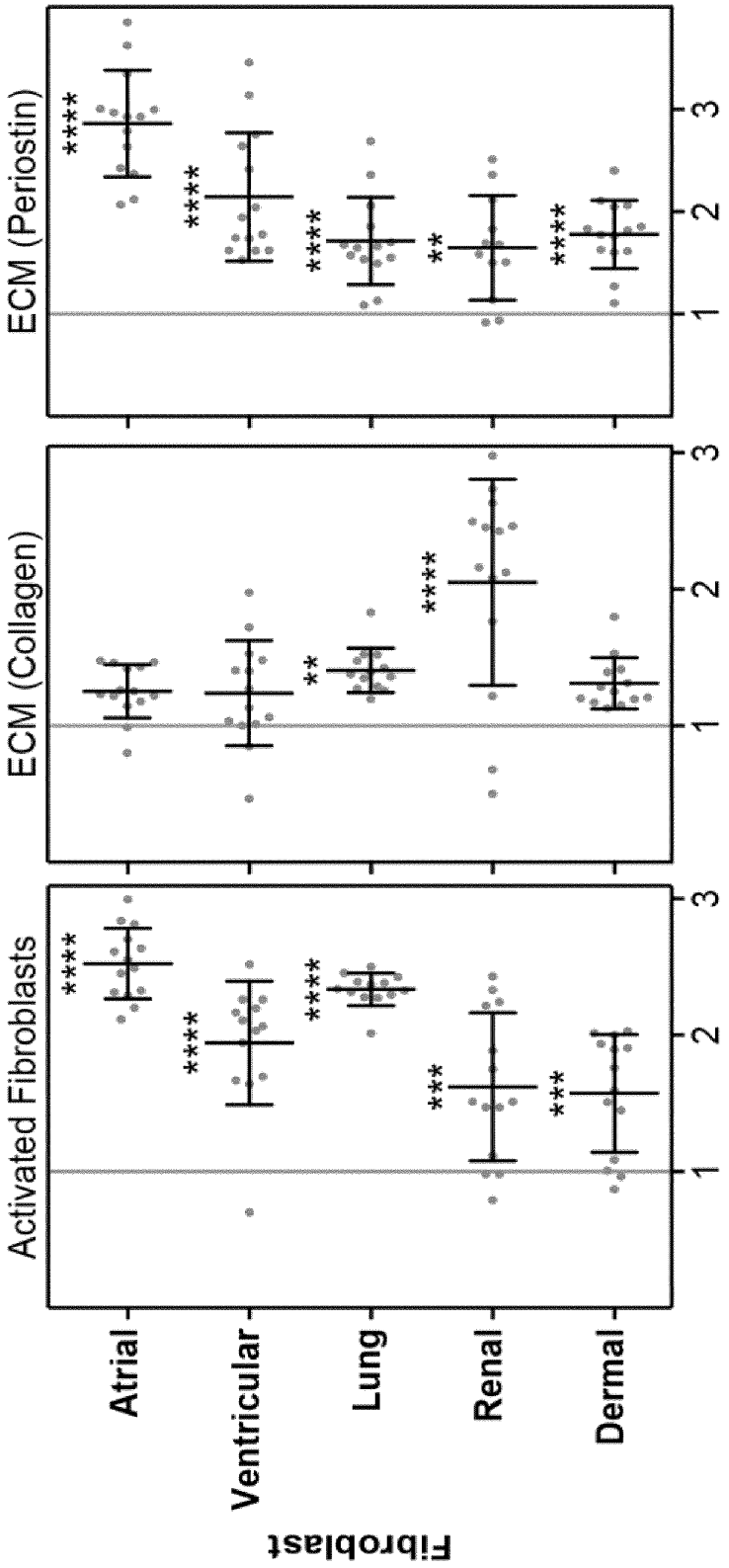
Activated Fibroblasts
ECM (Collagen)
ECM (Periostin)
Atrial
Ventricular
Lung
Renal
Dermal
Fibroblast
1
2
3
****
***
**

Figure 21A

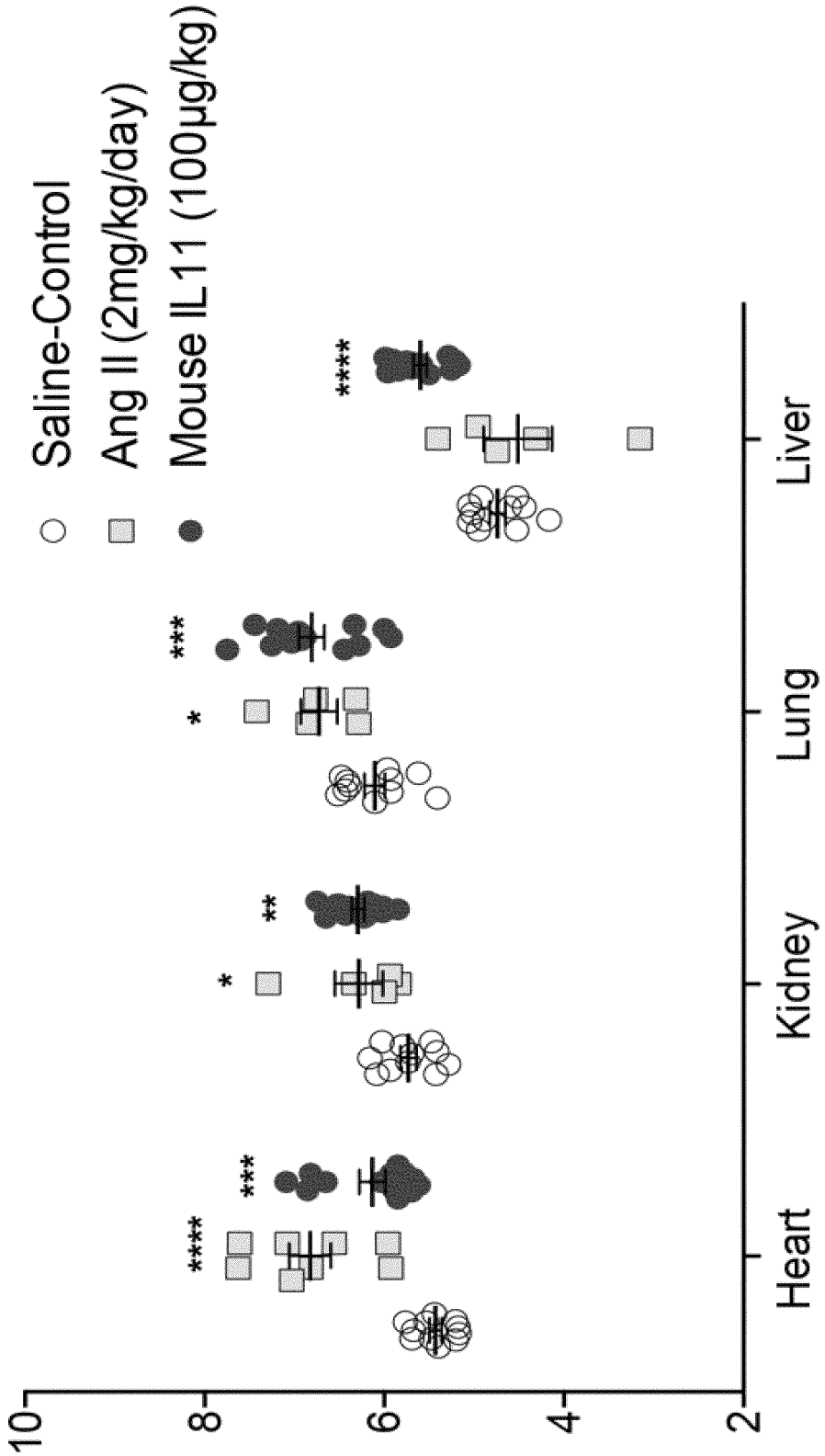
Saline-Control
Ang II (2mg/kg/day)
Mouse IL11 (100µg/kg)
10
8
6
4
2
Heart
Kidney
Lung
Liver
****
***
**
*
*
***
*
****

Figure 21B

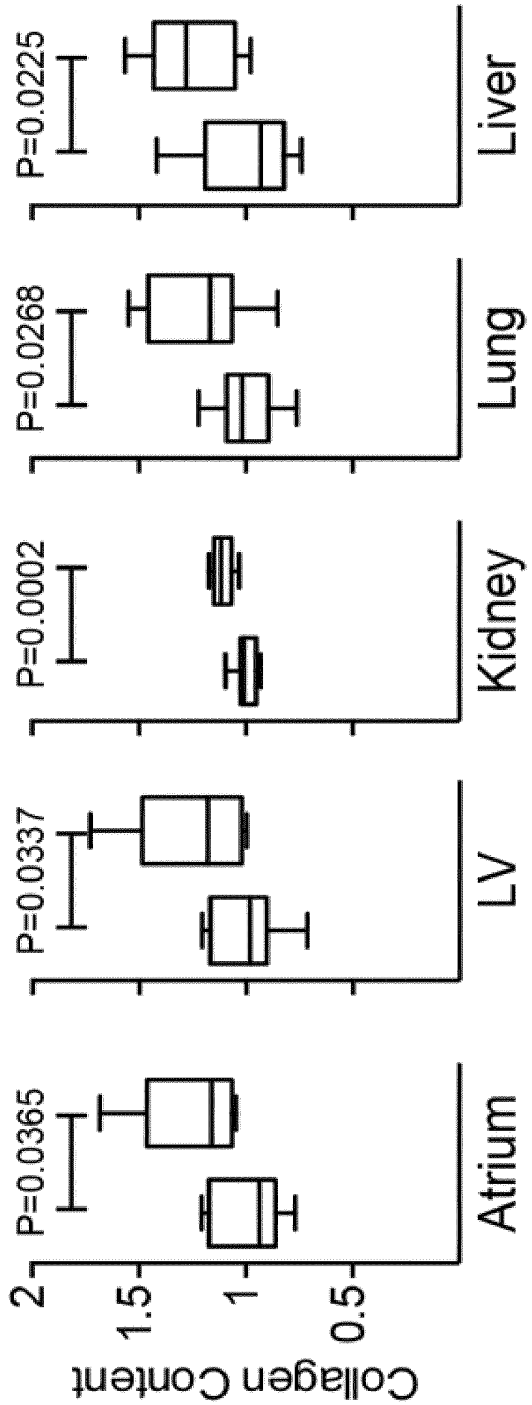
Collagen Content
2
1.5
1
0.5
P=0.0365
P=0.0337
P=0.0002
P=0.0268
P=0.0225
Atrium
LV
Kidney
Lung
Liver

Figure 21C

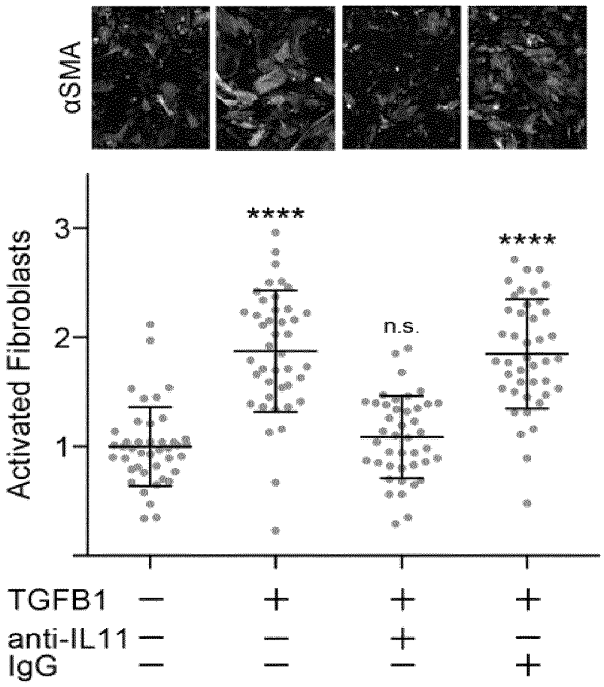

Figure 22A

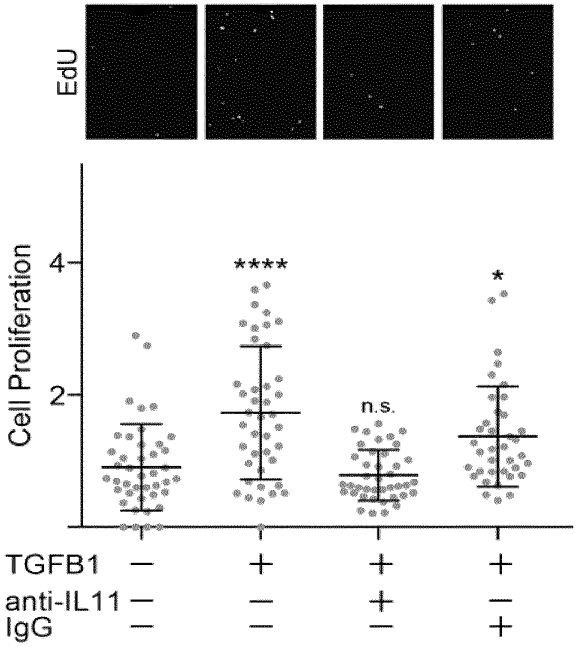

Figure 22B

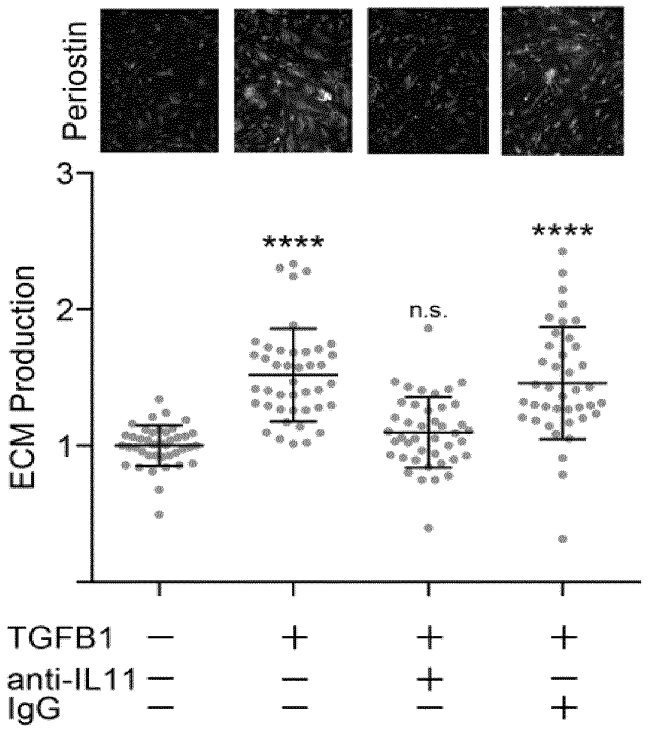

Figure 22C

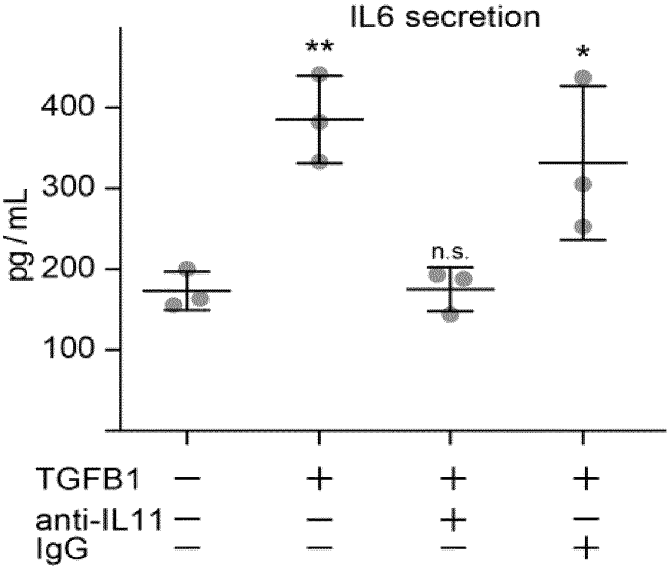

Figure 22D

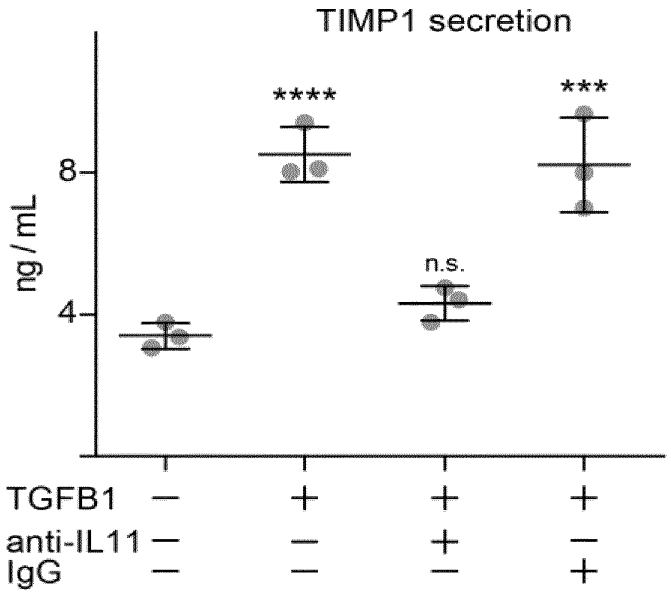

Figure 22E

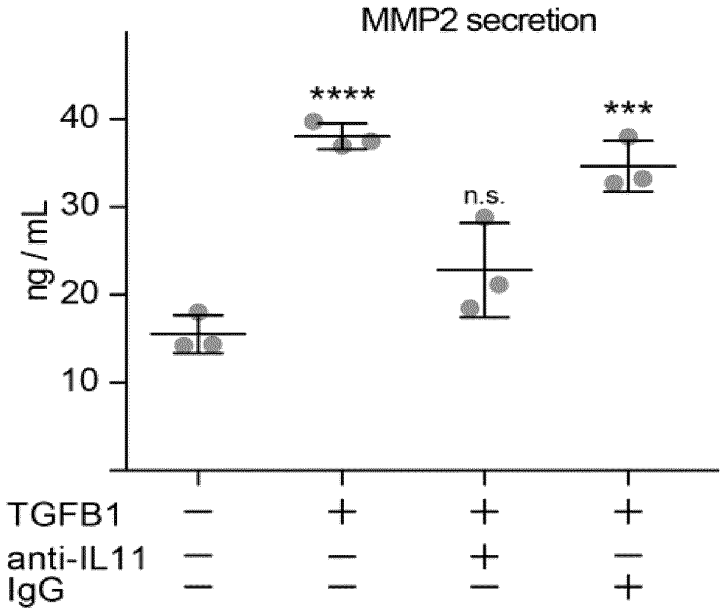

Figure 22F

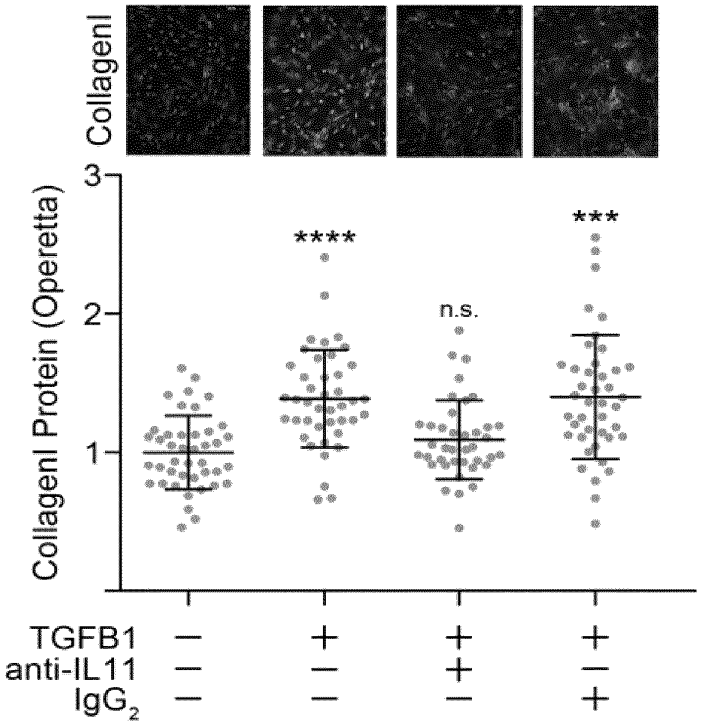

Figure 23A

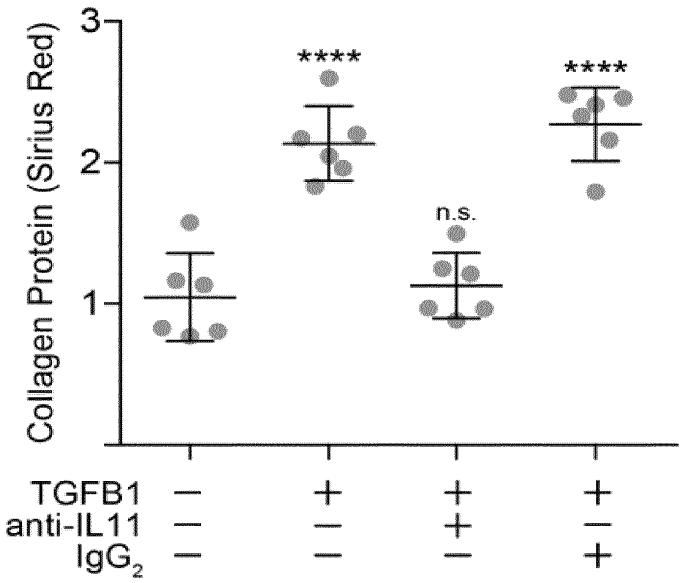

Figure 23B

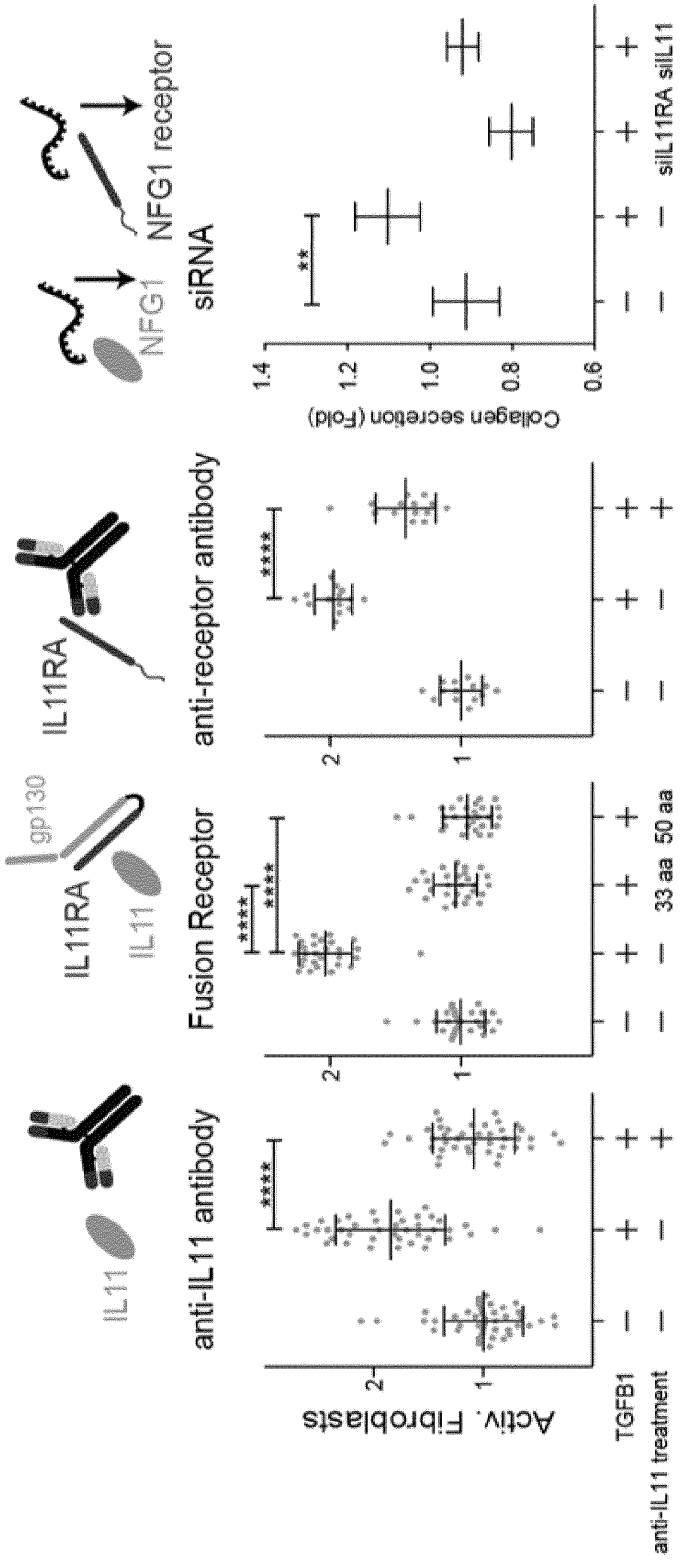
IL11
anti-IL11 antibody
gp130
IL11RA
IL11
Fusion Receptor
IL11RA
anti-receptor antibody
NFG1
NFG1 receptor
siRNA
Activ. Fibroblasts
Collagen secretion (Fold)
TGFB1
anti-IL11 treatment
33 aa
50 aa
linker between gp130 & IL11RA
siIL11RA
siIL11

Figure 24

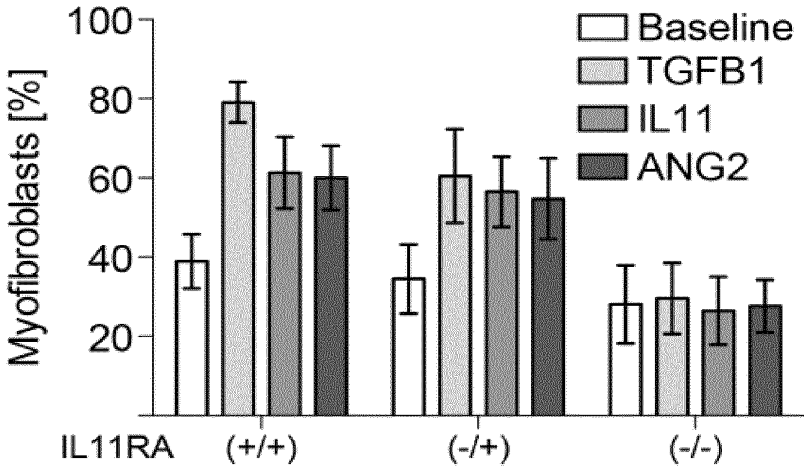

Figure 25A

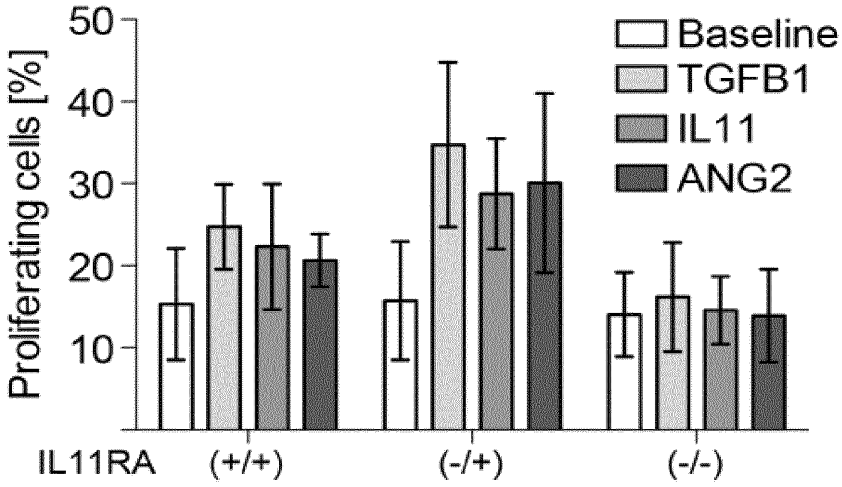

Figure 25B

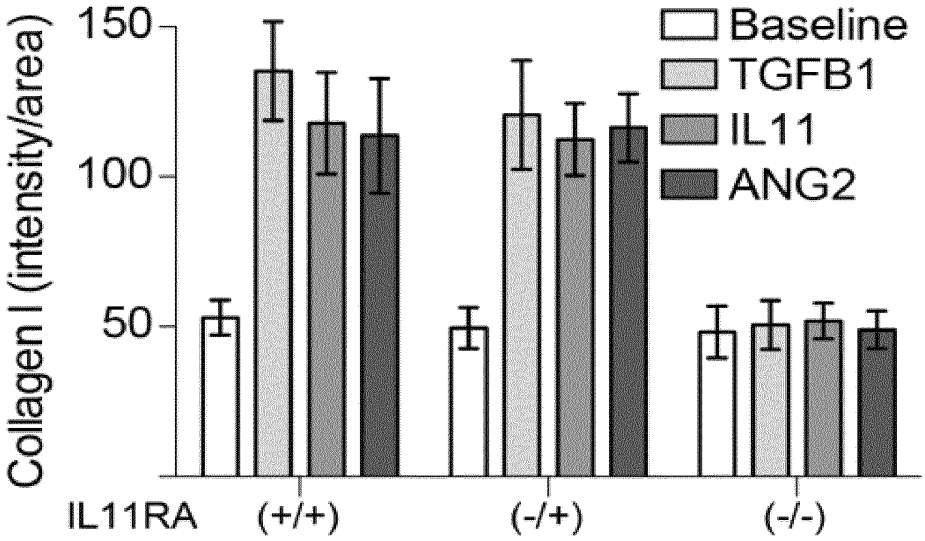

Figure 25C

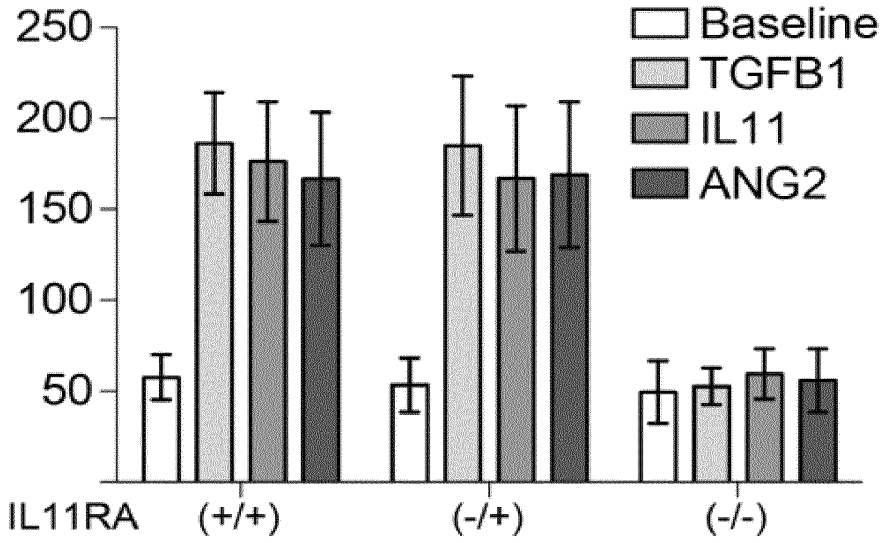

Figure 25D

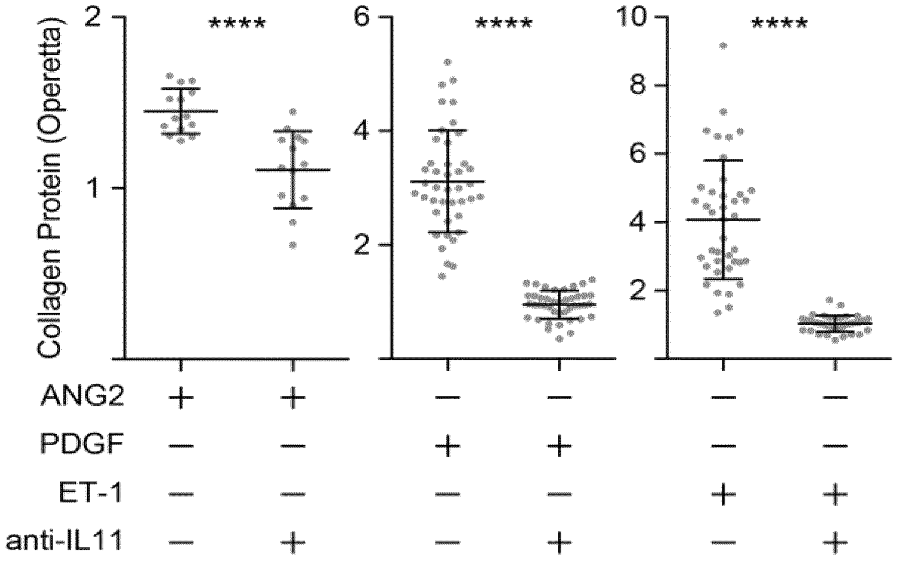

Figure 26A

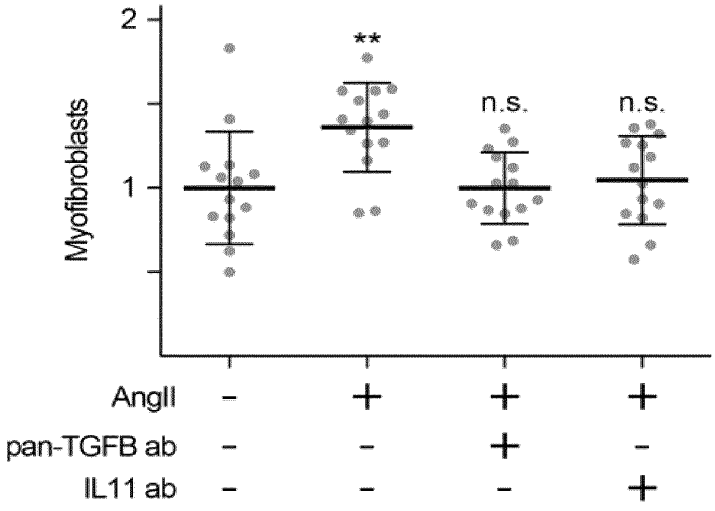

Figure 26B

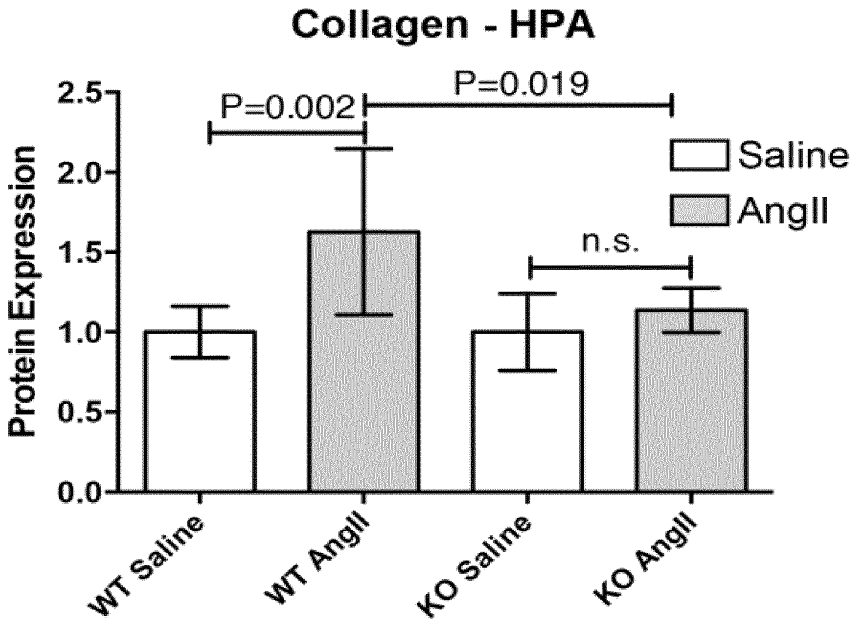

Figure 27A

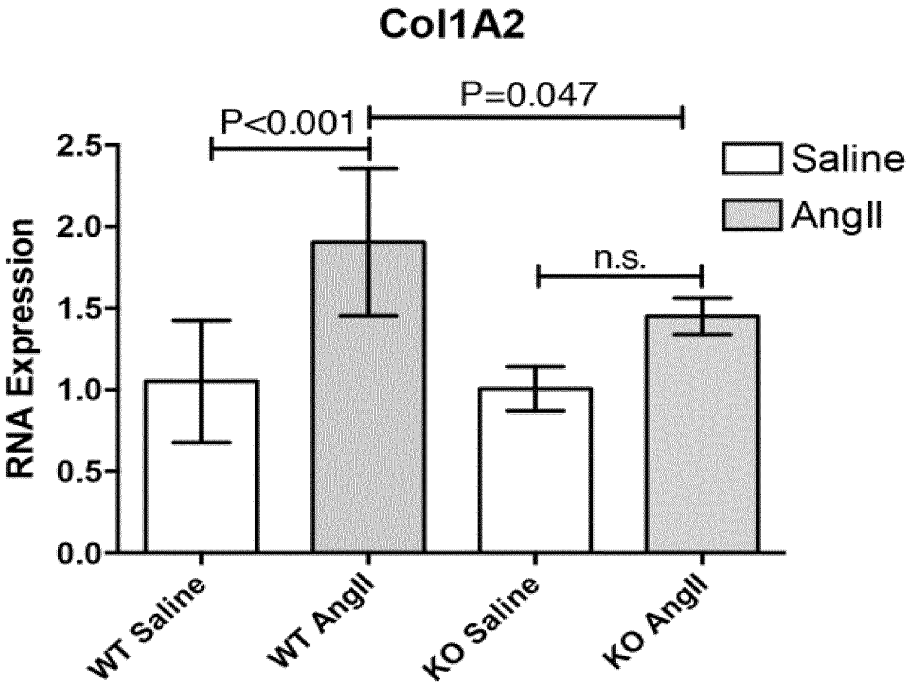

Figure 27B

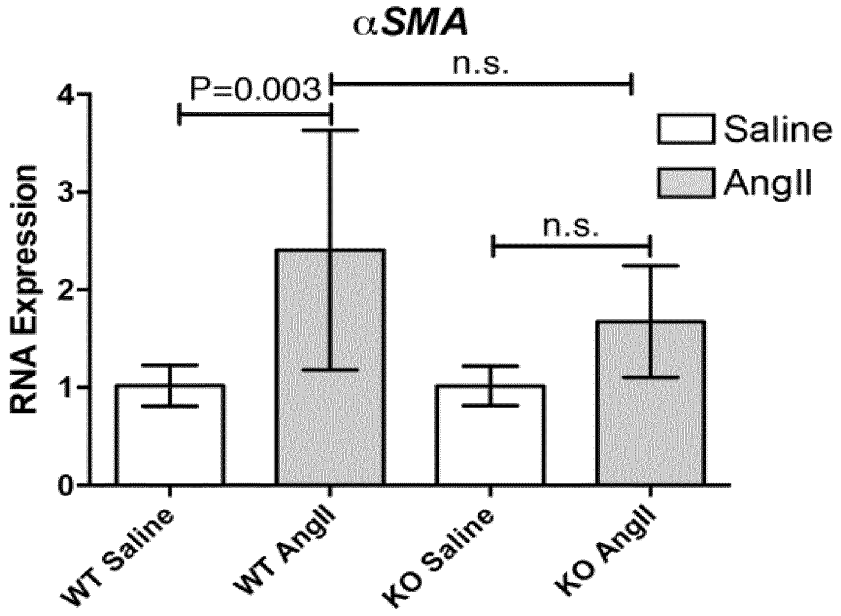

Figure 27C

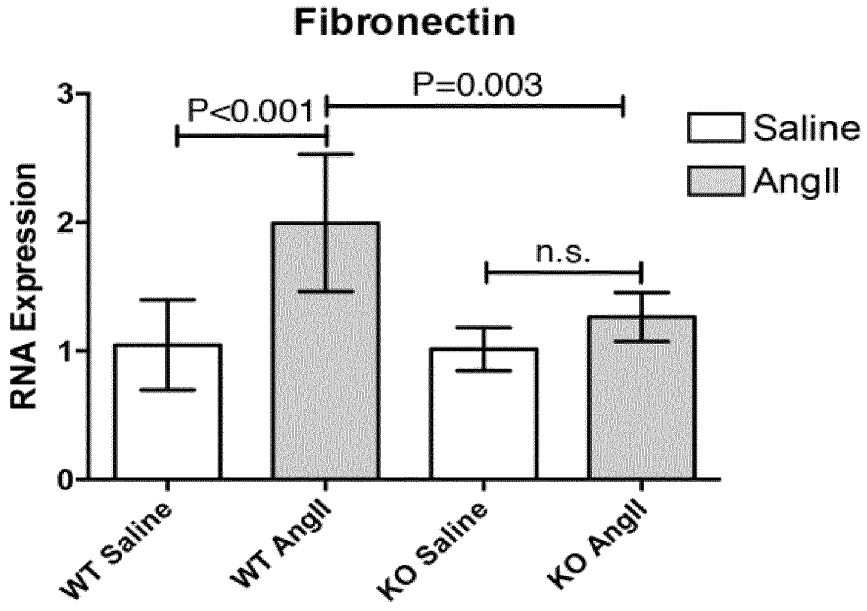

Figure 27D

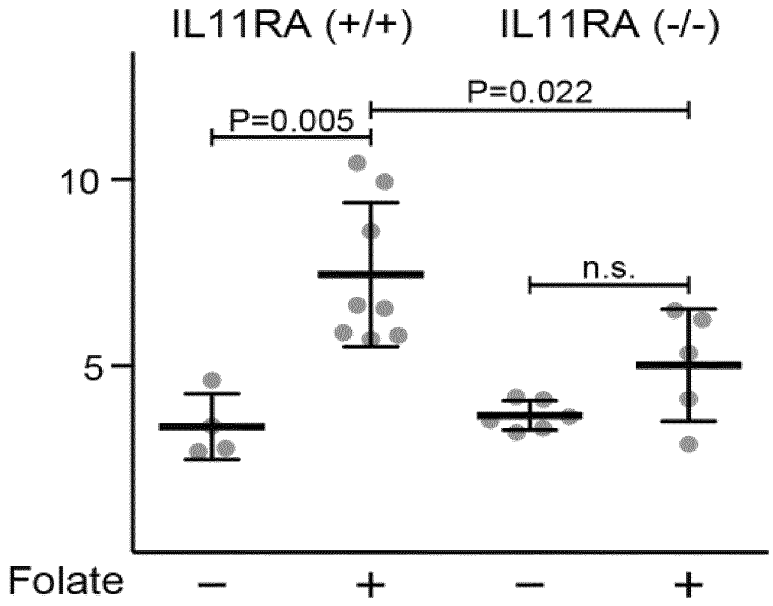

Figure 28

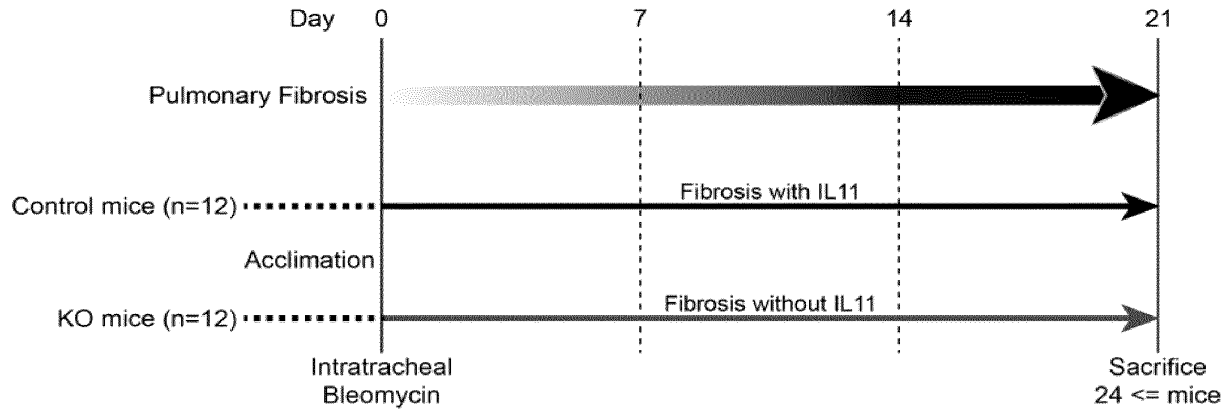

Figure 29A

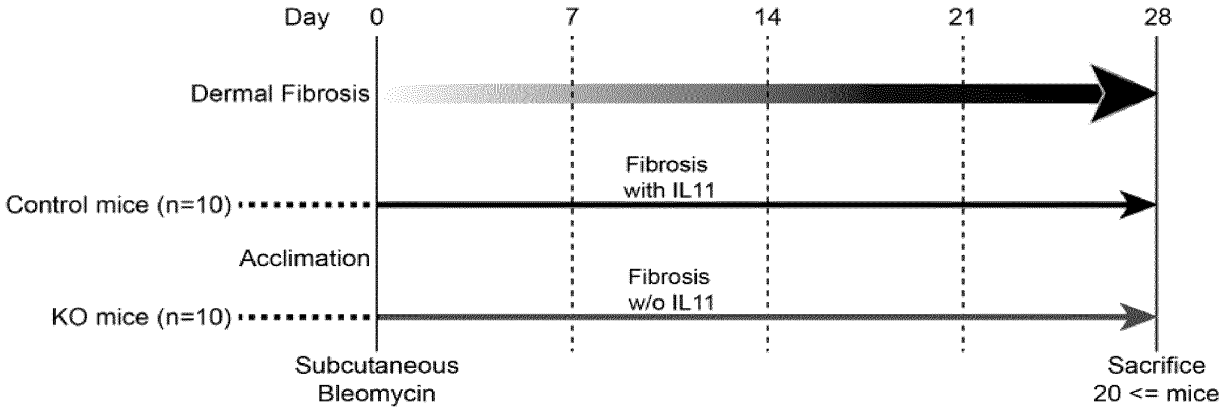

Figure 29B

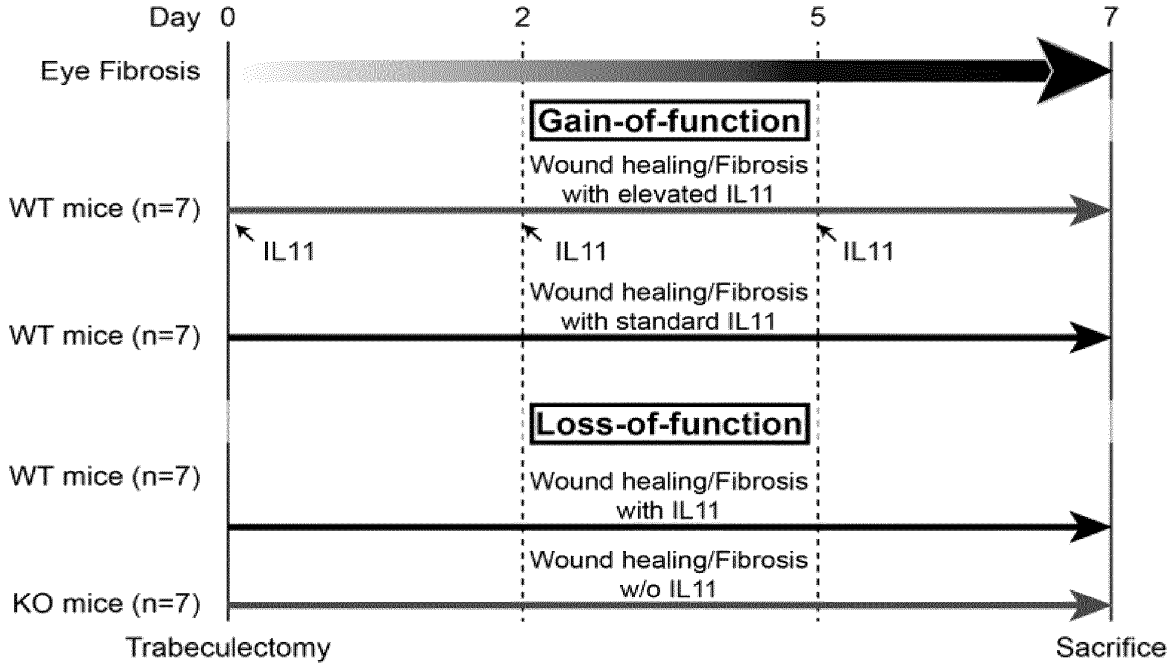
Day 0
2
5
7
Eye Fibrosis
Gain-of-function
Wound healing/Fibrosis with elevated IL11
WT mice (n=7)
IL11
IL11
IL11
Wound healing/Fibrosis with standard IL11
WT mice (n=7)
Loss-of-function
WT mice (n=7)
Wound healing/Fibrosis with IL11
Wound healing/Fibrosis w/o IL11
KO mice (n=7)
Trabeculectomy
Sacrifice

Figure 29C

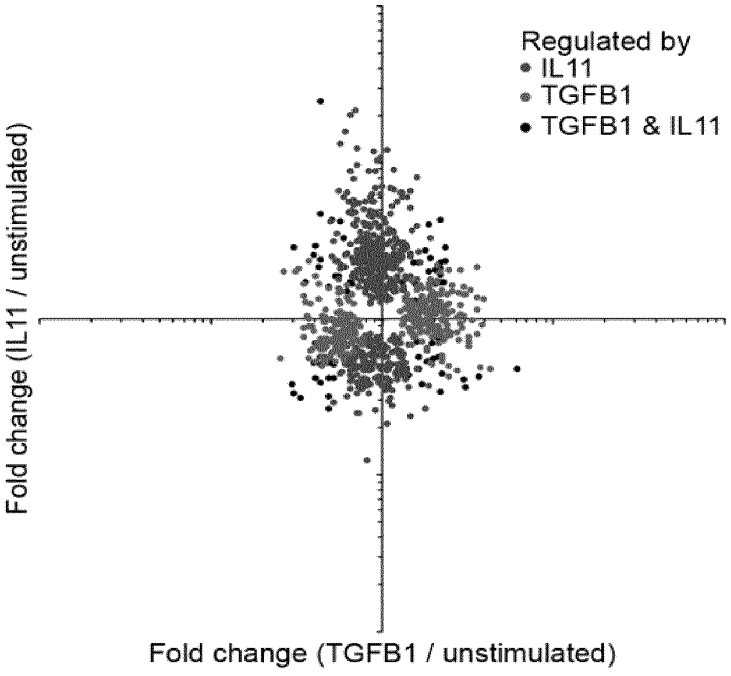

Figure 30A

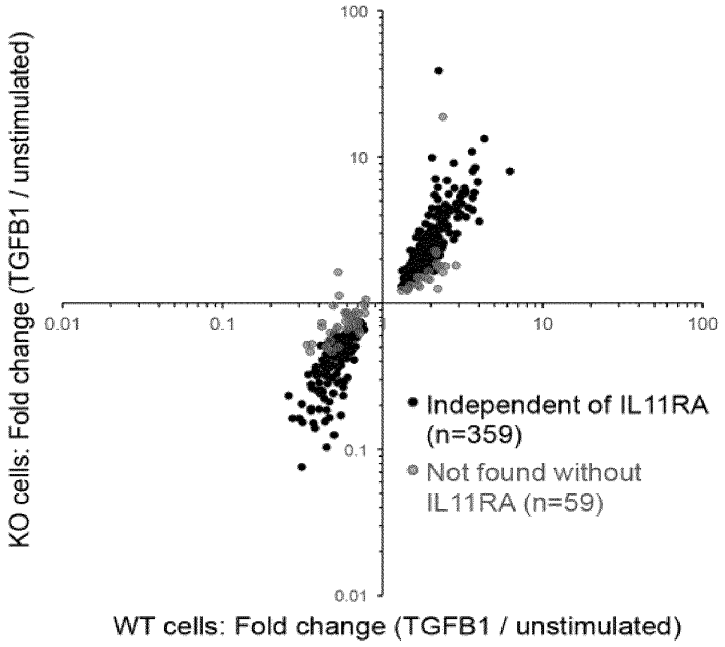

Figure 30B

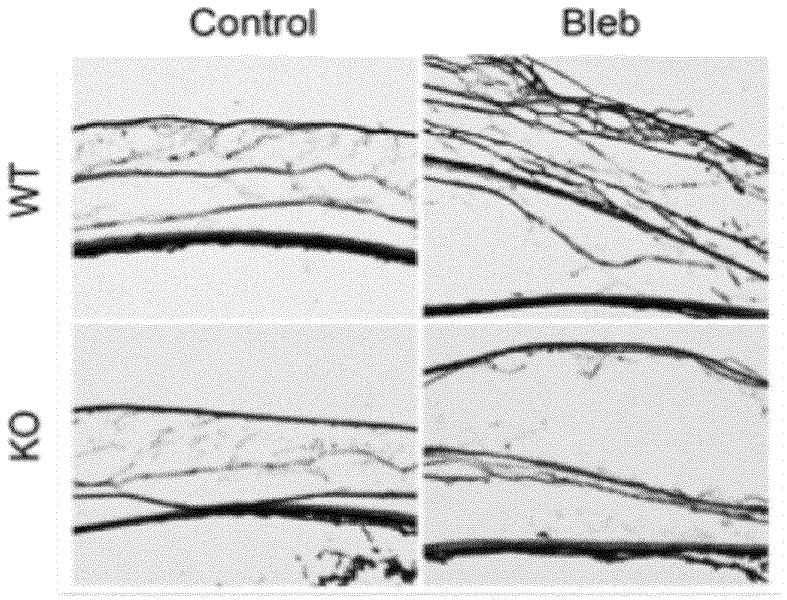

Figure 31A

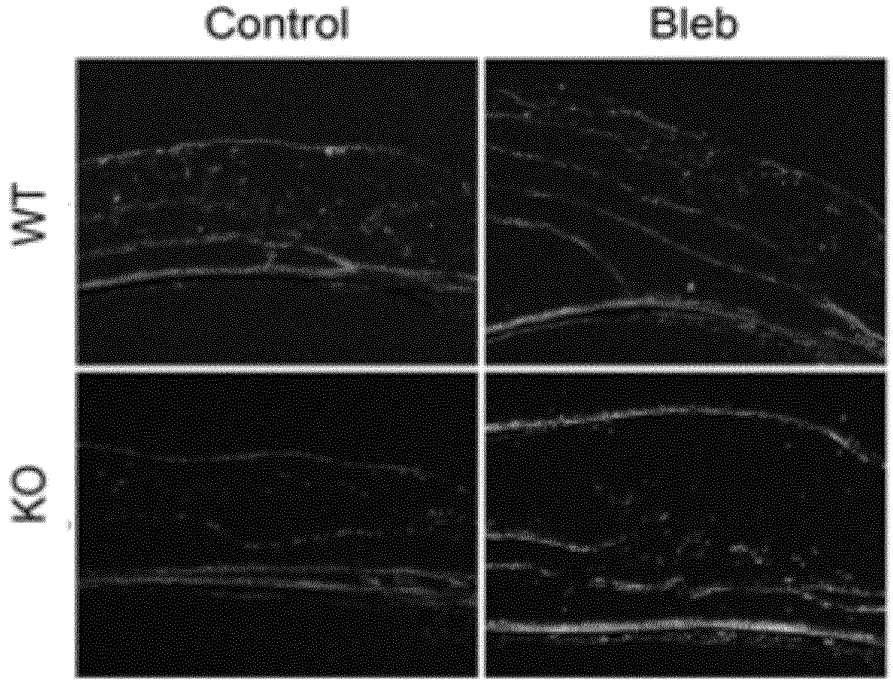

Figure 31B

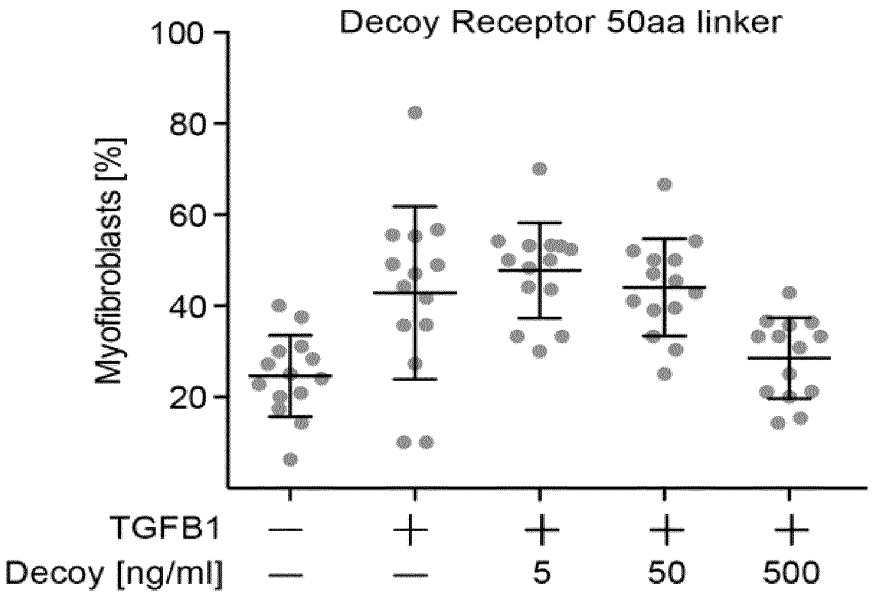

Figure 32A

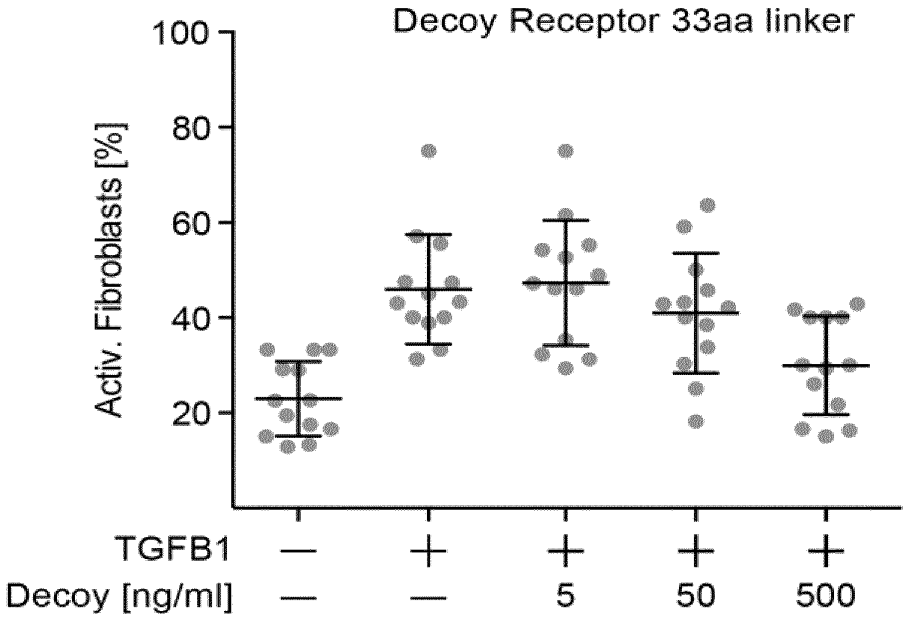

Figure 32B

SNP ID	Position (hg19)	P value	FDR	Genotype	Minor Allele	Sequence	SEQ ID NO
rs10831850	11-12566917	1.76E-07	0.0248	G/A	A	GTAAGGGATGTGAATCGGGTACTGA[A/G]GAAAGAG CCTGGATGCAGAGCCAGC	19
rs4756936	11-12599138	1.76E-07	0.0248	C/T	T	TTGATAACTTCAGCATCTGGATCAC[C/T]GTGGGATT AGCATCTGTTTGTATTT	20
rs6485827	11-12578182	1.76E-07	0.0248	C/T	C	GTGTGATTGCTTAAAAAAAACTACT[C/T]ACATTGTTT TGAATCACACCTCACA	21
rs7120273	11-12581045	1.44E-07	0.0248	T/C	T	GCTCAGCTAATCAATGACCAGTCTC[C/T]TTAATTCTT CTAATGCCTATATGGT	22
rs895468	2-223621273	2.70E-07	0.0306	G/A	G	GCAGTGCTCAGAAGAGCAGCAGCCA[A/G]TGACATT TTGGGGCTATAAGAGGTA	23

Figure 33

SNP ID	Position (hg19)	P value	FDR	Genotype	Minor Allele	Sequence	SEQ ID NO
rs1000574	19-55114775	0.0432	0.6581	C/T	T	TGTCCAGTAAATACTTAACATTTTA[C/T]GTGCA ATGTATGTCATAAATATGGG	24
rs10403345	19-56741808	0.0355	0.6372	G/A	G	GGgtgaagtttggaaacaggtatac[A/G]ttgtgatgcaatcgtca gaaccaag	25
rs10419994	19-55726220	0.0334	0.6372	C/T	T	aaaccataGTATCATCCTTCCCAAA[C/T]AGTCAAC CCAGggaatcacagagat	26
rs10426177	19-56269096	0.0103	0.6372	C/T	T	TAAGACGCTATTCTCTAATTCTGAA[C/T]GGAAG AACTCCTCTCCCAAGACATG	27
rs11084337	19-54916104	0.0370	0.6372	C/T	C	aggtggaacaacacaaagggtgggg[C/T]gaggcgtgcaattta aacattttct	28
rs11671244	19-56502995	0.0242	0.6372	C/A	A	TATTAGATTTTGTGTGGGATTTCAT[A/C]GTTAC ATTTGTTACCAGCCCAATTT	29
rs11882068	19-56227165	0.0005	0.2204	A/G	G	GATTCCAGTTCCAAGTCACATCATC[A/G]CCAG CTGGAAGACCTAGGGCAAAAG	30
rs12104147	19-54935505	0.0150	0.6372	G/A	A	ACCATGACGGTGTCCTCATTGCTTT[A/G]ACCAT TAGTAAtcattcattcattc	31
rs12608558	19-55715090	0.0275	0.6372	A/G	G	ACCTGTCACATTTTGTCAGCTCCCA[A/G]CCAC CCCCTACCACTGTCCTTATAA	32

rs12610132	19-55032293	0.0408	0.6372	A/G	G	GACACGATGTCTTCAGTCTCCAGCT[A/G]AGCT TGGACTGTGAGGATGGGTCAG	33
rs12610617	19-56443519	0.0030	0.3650	C/T	T	CTCTCAAGTTTGCCCAGGGGATACG[C/T]GGGA AGTGCCCCTGGGGGGCCGACC	34
rs160104	19-56433203	0.0012	0.2352	G/T	T	ctggtcttccagctctggtggct[G/T]ccagtatttcttgggttgtggc ctc	35
rs16986899	19-56549510	0.0265	0.6372	T/C	C	AACAAGGTGACAGACCAGGGAGTAA[C/T]GCCT CTCAGTGATGCCTTGAGAGTC	36
rs17815373	19-56608900	0.0483	0.7007	G/A	A	CGGCAGGCAGTAGGATGGACTGCGT[A/G]GAC GGCGGCCAGCATGTAAATGAAA	37
rs1895375	19-55035001	0.0329	0.6372	T/C	C	AAGTAAGGTGTCAAGGAGGCCATGC[C/T]CACT CTGTAGGTTCTAGGAAAGAAT	38
rs2043690	19-56383651	0.0305	0.6372	T/C	T	ATGCCTGAAAGAAACAAGAGCAAAT[C/T]GTCT CAGGAGGTAGGTAATAGGATG	39
rs2194	19-56257259	0.0064	0.5195	G/A	A	AGCATATTCATTGATTTCCTTACAT[A/G]CAAAT GCTCCTTTTTAAGTGCTCAA	40
rs2288419	19-55693244	0.0140	0.6372	A/G	G	TCAGTACGTATTCCTGCATCAGTGC[A/G]TCCT GCGGTTCCTCCAACAGTCAGC	41
rs2288521	19-55708557	0.0262	0.6372	T/C	T	AGTGGAGGCCCTGGAACCCGGGACG[C/T]TGT ACAATTTCACCGTGTGGGCAGA	42
rs2288527	19-55699077	0.0454	0.6738	A/G	G	TGCCATATAATCTCAGGGTGCAACG[A/G]ATAA ACAAGGGGTGATGCCGAAGAA	43

rs2637107	19-56566741	0.0317	0.6372	G/A	A	CTCTGTCCCCTCAACTTCTTTCTAC[A/G]TGGTC ATGTCCCTTCTTTAGTTCCT	44
rs299164	19-56319796	0.0273	0.6372	A/C	C	GCAACAAAAATCTTATACATCACCA[A/C]ATGTC TGCTTAGCGGCAGAATTGCC	45
rs299169	19-56316796	0.0324	0.6372	G/A	G	GCTAGGTAAAGGACTCTGAAAATAC[A/G]GCAA CATGGAAAACATCCAGTCTCC	46
rs303997	19-56424443	0.0007	0.2204	T/C	T	TCCATTTGCCCAGTGCAGCATAGCC[C/T]GCAT TGCCAAGGTGGTCTTCCCAAC	47
rs304001	19-56423668	0.0015	0.2352	C/T	C	CGTTTAACAAAGAAGACACTGAGAT[C/T]GAGG GCCTGGAAGTGCCTTTCATTG	48
rs304002	19-56423254	0.0141	0.6372	C/T	C	TTCTACGACTTTTTCACTGCCTACA[C/T]GAGTC CCAGGAGGAAGACTTCACAA	49
rs306463	19-56497077	0.0068	0.5195	T/G	T	gccaaatgtgtttgaaaattccatt[G/T]gaagaatttatggtgaatgc atttt	50
rs310445	19-56173551	0.0396	0.6372	G/A	G	GAAGATTGTTCAAGAAAAGGCAGAG[A/G]GCAT GATGACAACACACAAAATGAAGA	51
rs3745429	19-54937593	0.0233	0.6372	A/G	G	CCTGGGACTATCCCCTGGCCGGGCC[A/G]CAC ACATGTGCCCTGTGACCAGGGA	52
rs3786867	19-55684200	0.0394	0.6372	C/T	T	GCTGGCTGTGAGGAGTCCGCGAGAA[C/T]TCC CTTGCTTGTCCATGAATTTATC	53
rs4629084	19-56420157	0.0243	0.6372	G/T	G	AAGGTGAAGAGTGGGAAAAGGCAGA[G/T]GATC AGGAAAAATAACTAATGGGTA	54

rs464765	19-56430224	0.0208	0.6372	A/C	A	ggcttaataaaaggaattagagagc[A/C]ctccctcctctccaacat cttttc	55
rs4801278	19-56246520	0.0268	0.6372	G/A	A	TGGTCTGTGCTCTCCTTGCACACTC[A/G]TCTGT GGACATCACAGGAGGGAACA	56
rs4801635	19-56372705	0.0352	0.6372	C/T	C	GTGCGTATATGTGTTACAAGTGGCT[C/T]GTGTT GACCGCCTGCCTGTGGAAAG	57
rs516022	19-56609371	0.0408	0.6372	C/A	C	GCCTCGGGTGCTGACCGGGGGTGCC[A/C]TTG CTGGGCTTAGCAGGCCGGGCTT	58
rs6509882	19-55030183	0.0400	0.6372	A/G	G	TGATGTGCCACATCCTGTATAGGAA[A/G]CAGG TGATGTGGAAATGAGTCAGAC	59
rs6509883	19-55030376	0.0378	0.6372	A/G	G	CATGAAAACCCCTCAGATGGTACCA[A/G]AAAT ATAGACAATTGATCCAGAGAG	60
rs6509933	19-55683104	0.0188	0.6372	T/C	T	gtcccagctactcacacaggaggat[C/T]gcaggagtttgaaacc agcctgtgc	61
rs6509939	19-55851708	0.0337	0.6372	T/C	C	CACCCTCAGGGCCTCTCCCTGACCC[C/T]TCTC TCACCCCCGGGACCTCCCTGC	62
rs8110255	19-56568978	0.0045	0.4527	T/C	C	GCAGGTCTGTGTTCCTTGTTATGTT[C/T]CCTTG CTACAGGCACTCTCAGCCTT	63
rs8112791	19-56723492	0.0236	0.6372	C/A	C	gtcagtttgtgaacaggtaaaatca[A/C]aatgctcatgttctctaca gggaaa	64
rs873732	19-56745520	0.0194	0.6372	G/A	A	AGCCAATTCGTCAGTGAATGAGGCA[A/G]AGAA ATTGGTAAAAAGAAGGAAAGT	65

Figure 34

SNP ID	Position (hg19)	P value	FDR	Genotype	Minor Allele	Sequence	SEQ ID NO
rs2823699	21-17628689	1.62E-08	0.0033	T/C	T	TACATAGTAGGCTTAAGAGCAAATG[C/T]CTACC TTTTCCTCTGTTTTCAACTC	66
rs4973978	3-41667545	1.69E-08	0.0033	T/G	G	GTTGGTTGGTTTGTTTCCCCTTTAA[G/T]GGTGC CATTTAATGACAGATTTCAT	67
rs11744285	5-124622296	1.75E-08	0.0033	A/G	G	GCCCCGGTGACAAGAATGGCAAAAC[A/G]TTTA TTCGGCATTAACAATGTGTAA	68
rs7120273	11-12581045	3.32E-08	0.0041	T/C	T	GCTCAGCTAATCAATGACCAGTCTC[C/T]TTAAT TCTTCTAATGCCTATATGGT	69
rs10831850	11-12566917	5.12E-08	0.0041	G/A	A	GTAAGGGATGTGAATCGGGTACTGA[A/G]GAAA GAGCCTGGATGCAGAGCCAGC	70
rs4756936	11-12599138	5.12E-08	0.0041	C/T	T	TTGATAACTTCAGCATCTGGATCAC[C/T]GTGG GATTAGCATCTGTTTGTATTT	71
rs6485827	11-12578182	5.12E-08	0.0041	C/T	C	GTGTGATTGCTTAAAAAAAACTACT[C/T]ACATT GTTTTGAATCACACCTCACA	72

rs7079768	10-131271870	9.92E-08	0.0070	G/A	A	ACTTGTGCCAGGCTGGCTTTGCAAC[A/G]ATGA GCCTGAGAAGCTGTTAGAAGT	73
rs1293764	12-113425679	1.27E-07	0.0080	A/G	A	GAGACACAAGAGGTGGGCAGGTCTT[A/G]GGG ATTTAGGAGTTGGGTTCAAGGC	74
rs1862945	7-50936184	6.84E-07	0.0387	G/A	A	TGAGTCTGTGAGGAGAAATGAACAA[A/G]TCTA CCACAGTCATCCAGAATGAGA	75
rs10496038	2-55340963	9.41E-07	0.0484	C/T	T	ATCAGAAGGCTAAGGAACCACCTGT[C/T]TAATA GTCTGGTGCCAAACACAGGC	76
rs12669489	7-50961697	1.05E-06	0.0493	G/A	A	catcaataagaaaaacaaaaataat[A/G]taatagaaaaatgcat aagagactt	77

Figure 35

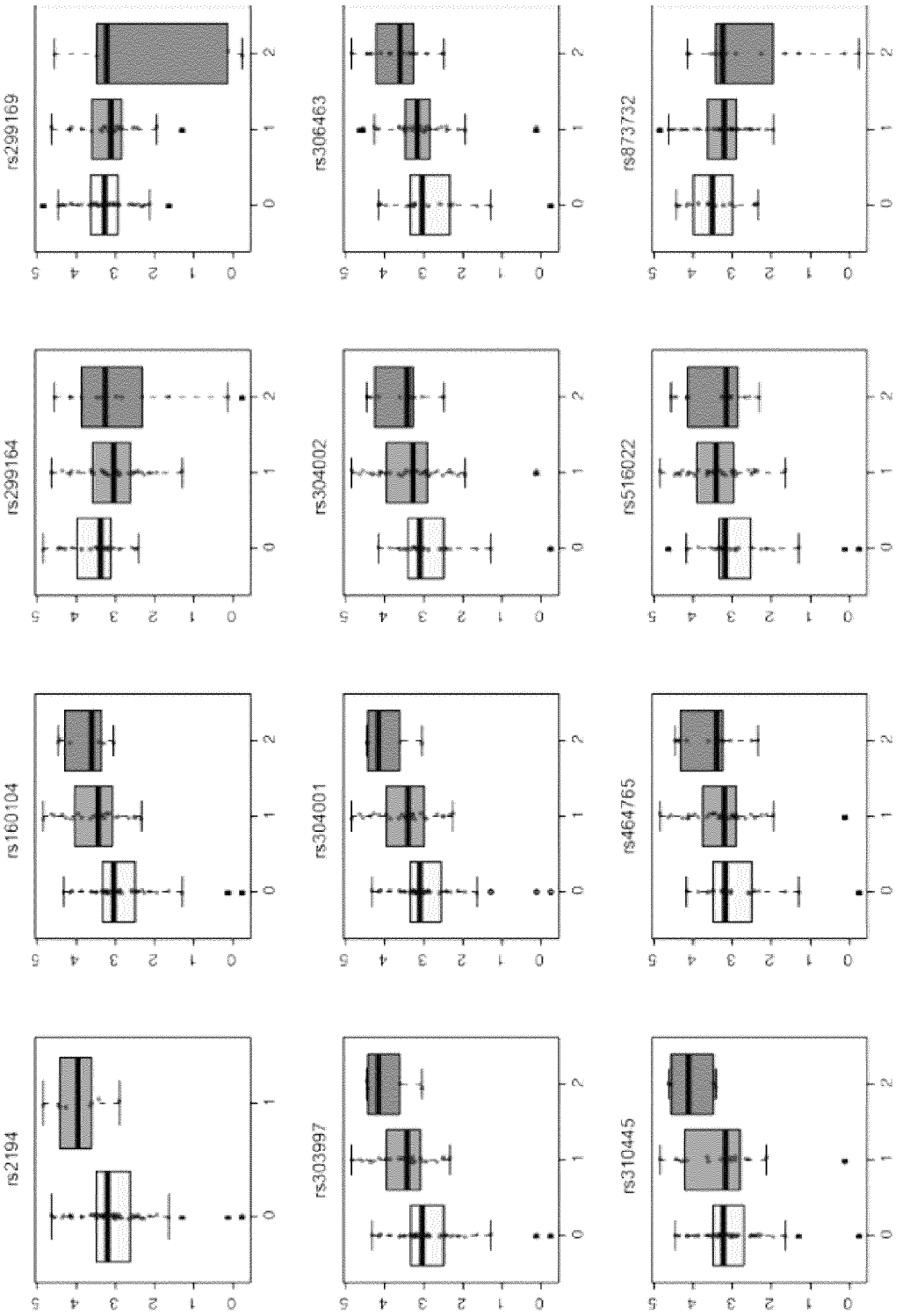
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Figure 36A

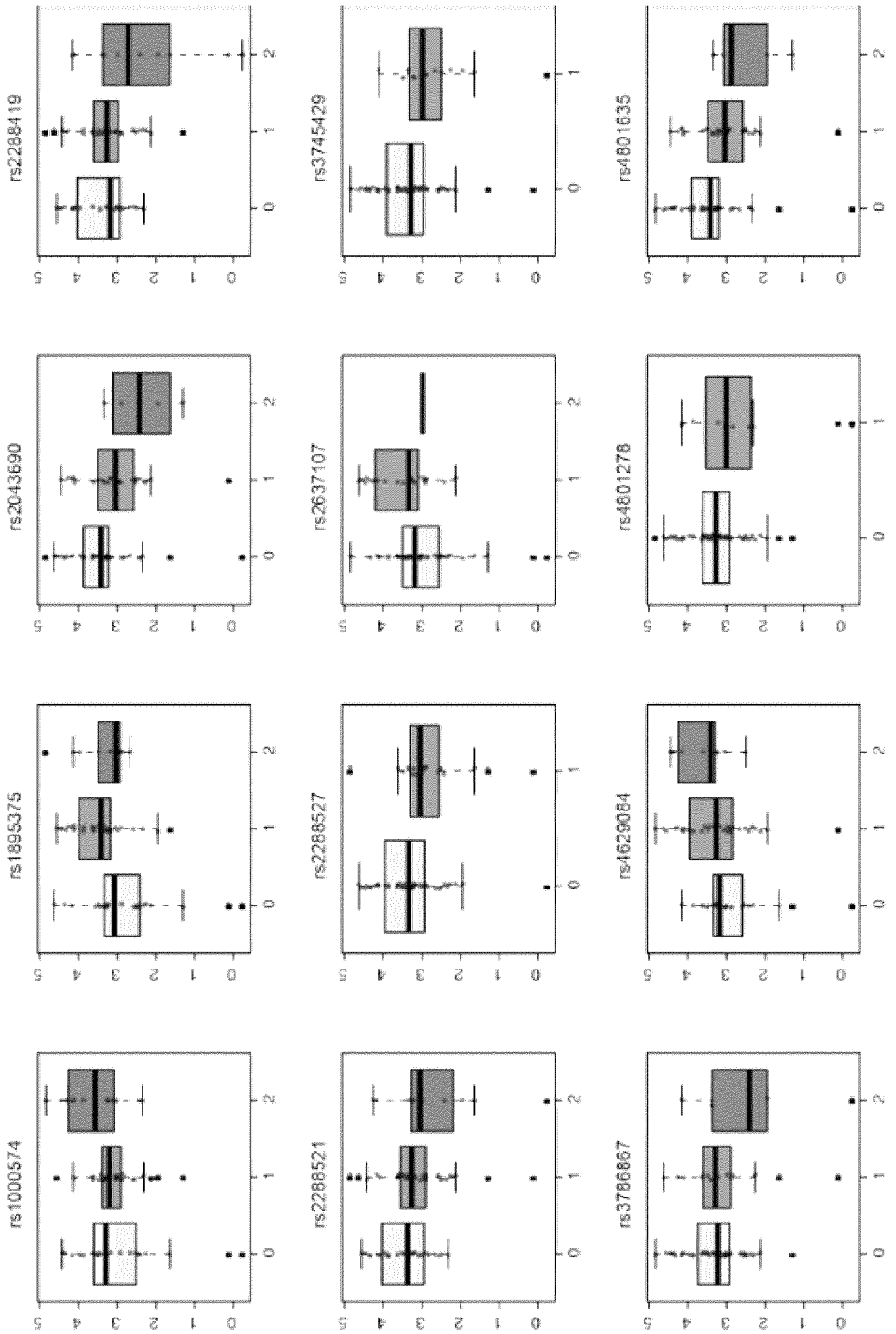
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Figure 36B

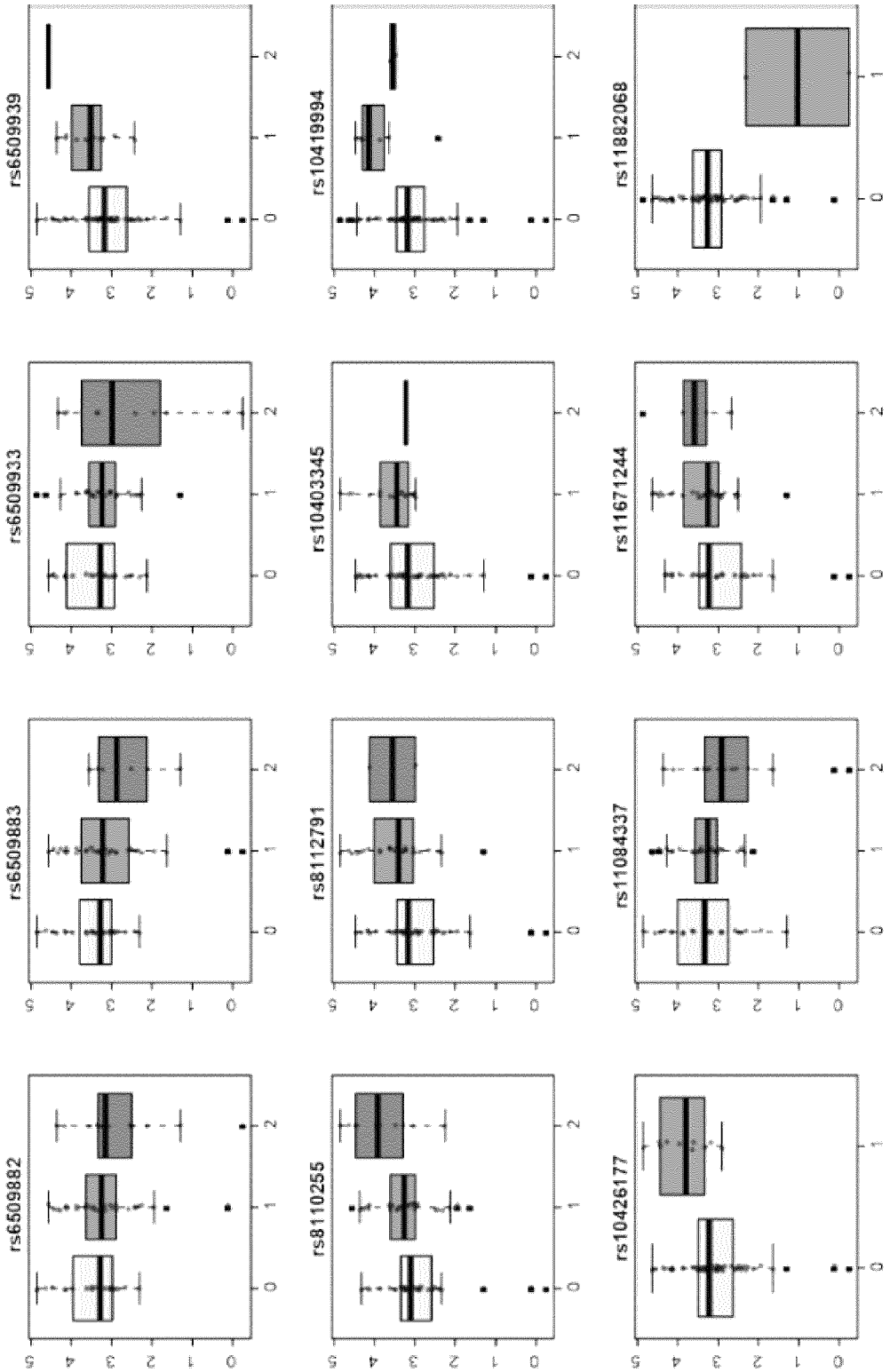
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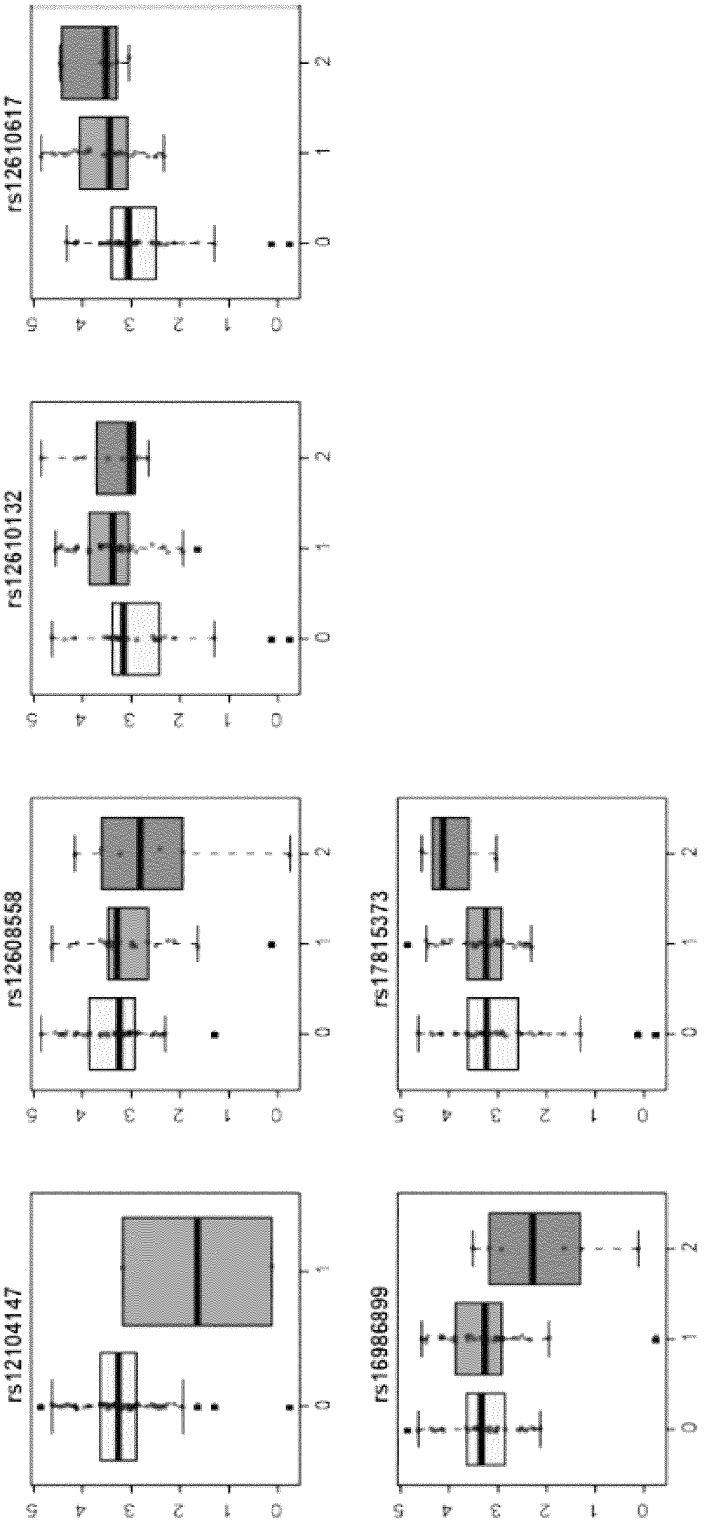
Figure 36C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rs17815373
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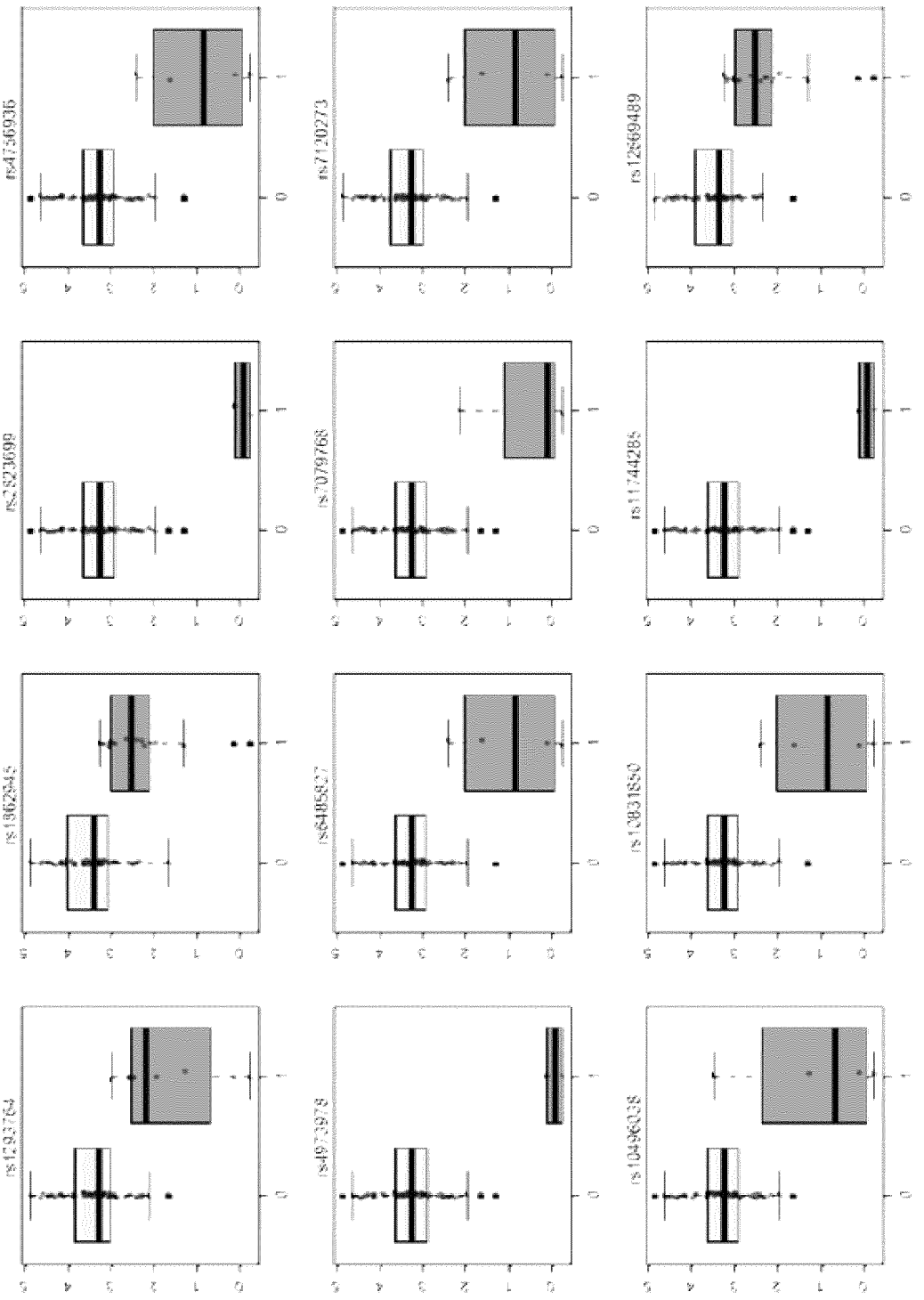
rs16986899

Figure 36D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rs12831850
rs4973978
rs10496038
rs1293764

Figure 37

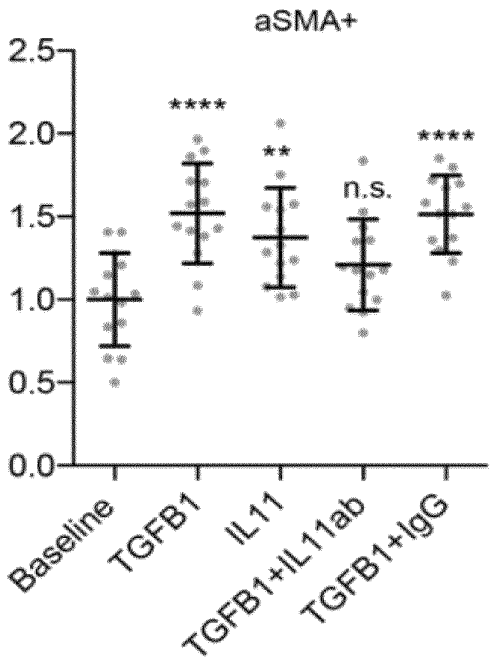

Figure 38A

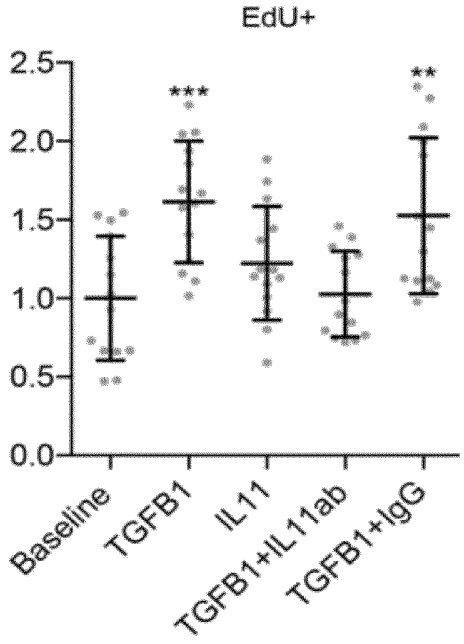

Figure 38B

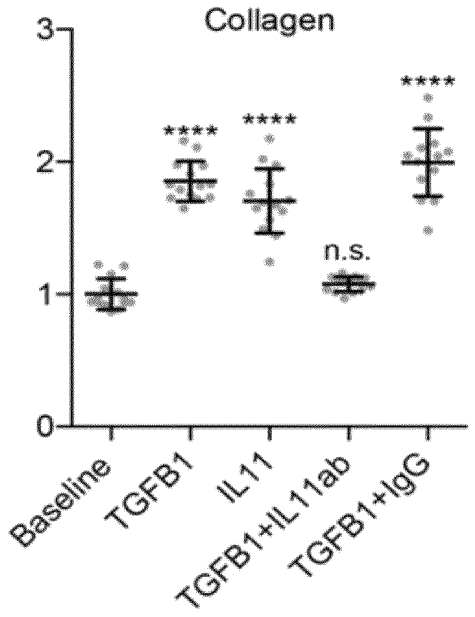

Figure 38C

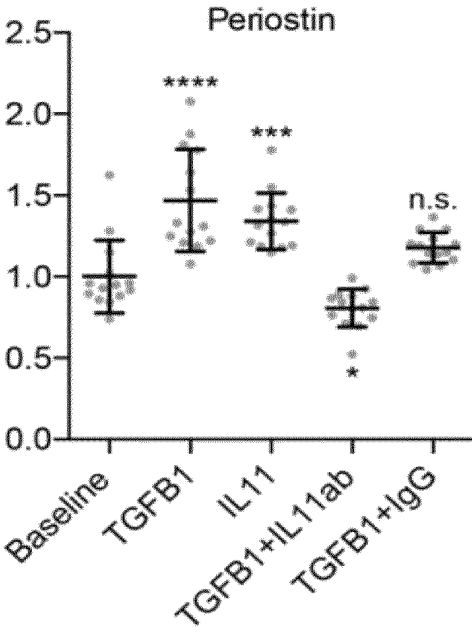

Figure 38D

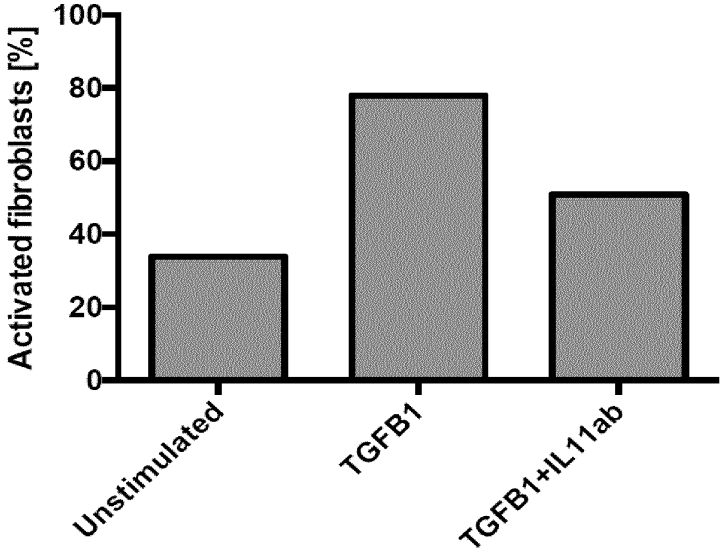

Figure 39

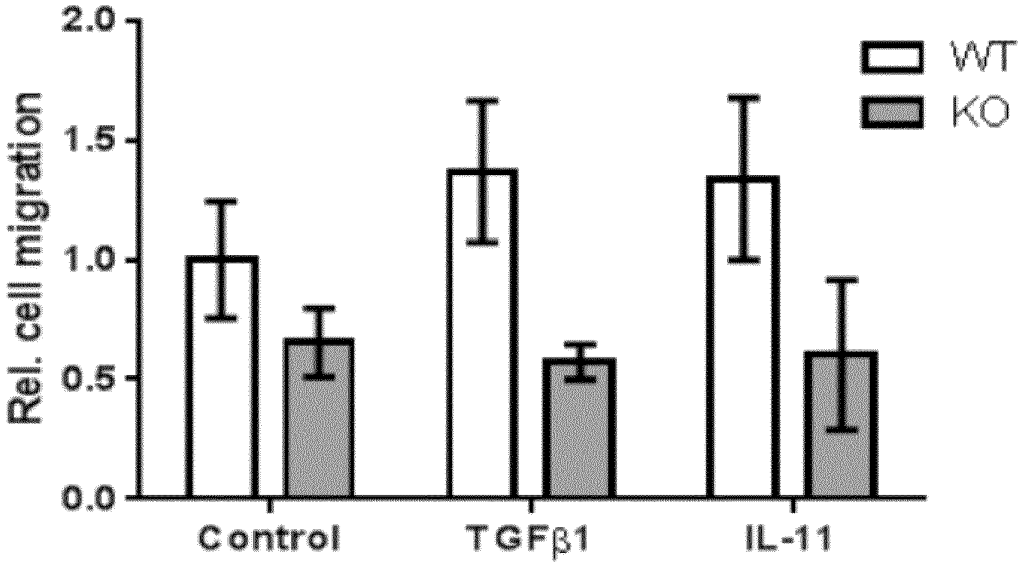

Figure 40

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/081430

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/24 C07K16/28 A61K39/00
ADD.

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 C12N A61K

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)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	M. OBANA ET AL: "Therapeutic Activation of Signal Transducer and Activator of Transcription 3 by Interleukin-11 Ameliorates Cardiac Fibrosis After Myocardial Infarction", CIRCULATION, vol. 121, no. 5, 9 February 2010 (2010-02-09), pages 684-691, XP055359357, ISSN: 0009-7322, DOI: 10.1161/CIRCULATIONAHA.109.893677 page 685, left-hand column, paragraphs 1-3,5 page 686, right-hand column, paragraph 1 - page 687, right-hand column, paragraph 2; figure 2 page 689, left-hand column, paragraph 1 - page 690, right-hand column, paragraph 2 ----- -/--	1-34

[X] Further documents are listed in the continuation of Box C.

[X] 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
7 April 2017	18/04/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Page, Michael

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	M Stangou ET AL: "Effect of IL-11 on glomerular expression of TGF-beta and extracellular matrix in nephrotoxic nephritis in Wistar Kyoto rats", J Nephrol, 1 January 2011 (2011-01-01), pages 106-111, XP055359363, Retrieved from the Internet: URL:http://www.ncbi.nlm.nih.gov/pubmed/20640990 [retrieved on 2017-03-28] abstract page 106, right-hand column, paragraph 1-3 page 107, right-hand column, paragraph 4 - page 108, right-hand column, paragraph 1 page 109, left-hand column, paragraph 1 - right-hand column, paragraph 1 page 110, left-hand column, paragraph 2	1-34
A	US 2010/093976 A1 (AZUMA JUNICHI [JP] ET AL) 15 April 2010 (2010-04-15) paragraphs [0001], [0019], [0086], [0087]; claims 8,9; figure 10	1-34
A	AHROM HAM ET AL: "Critical Role of Interleukin-11 in Isoflurane-mediated Protection against Ischemic Acute Kidney Injury in Mice", ANESTHESIOLOGY., vol. 119, no. 6, 1 December 2013 (2013-12-01), pages 1389-1401, XP055359340, PHILADELPHIA, PA, US ISSN: 0003-3022, DOI: 10.1097/ALN.0b013e3182a950da page 7, paragraph 4 - page 8, paragraph 5	1-34
X	WO 98/36061 A2 (UNIV MANCHESTER [GB]; FERGUSON MARK WILLIAM JAMES [GB]; KANE SHARON O) 20 August 1998 (1998-08-20) page 6, paragraph 2 - page 11, paragraph 1; claims 1,5	1-34
X	WO 00/78336 A1 (GENETICS INST [US]; UNIV JOHNS HOPKINS [US]) 28 December 2000 (2000-12-28) page 1, lines 13,14 page 5, line 27 - page 6, line 32 page 15, lines 19-23; claims 13,14,17,21,22	1-34

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2016/081430

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2010093976 A1	15-04-2010	EP 2077116 A1	08-07-2009
		JP 5191393 B2	08-05-2013
		US 2010093976 A1	15-04-2010
		WO 2008050789 A1	02-05-2008
WO 9836061 A2	20-08-1998	AU 6220398 A	08-09-1998
		WO 9836061 A2	20-08-1998
WO 0078336 A1	28-12-2000	AU 5756100 A	09-01-2001
		WO 0078336 A1	28-12-2000

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

To
Clegg, Richard
MEWBURN ELLIS LLP
City Tower
40 Basinghall Street
London
Greater London EC2V 5DE
ROYAUME UNI

RECEIVED
18 APR 2017
MEWBURN ELLIS LLP

Date of mailing *(day/month/year)*	18 April 2017 (18-04-2017)
Applicant's or agent's file reference RIC/FP7230618	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No PCT/EP2016/081430	International filing date *(day/month/year)* 16 December 2016 (16-12-2016)
Applicant SINGAPORE HEALTH SERVICES PTE LTD	

1 [X] The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46)

When? The time limit for filing such amendments is normally two months from the date of transmittal of the International Search Report

How? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Fascimile No (41-22) 338 82 70

For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9 004 - 9 011

2 [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith

3 [ ] **With regard to any protest** against payment of (an) additional fee(s) under Rule 40 2, the applicant is notified that

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with any applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices

[ ] no decision has been made yet on the protest, the applicant will be notified as soon as a decision is made

4 **Reminders**

The applicant may **submit comments on an informal basis on the written opinion of the International Searching Authority** to the International Bureau These comments will be made available to the public after international publication The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established

Shortly after the expiration of **18 months from the priority date, the international application will be published** by the International Bureau If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90*bis* 1 and 90*bis* 3)

Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later), otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for **entry into the national phase** before those designated Offices In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months For details about the applicable time limits, Office by Office, see www wipo int/pct/en/texts/time_limits html and the *PCT Applicant's Guide*, National Chapters.

Within **19 months from the priority date, the applicant may request that a supplementary international search be carried out** by a different International Searching Authority that offers this service (Rule 45*bis* 1) The procedure for requesting supplementary international search is described in the *PCT Applicant's Guide*, International Phase, paragraphs 8.006-8 032

Name and mailing address of the International Searching Authority European Patent Office, P B 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel (+31-70) 340-2040 Fax (+31-70) 340-3016	Authorized officer LANGER, Monika Tel +49 (0)89 2399-8205

Form PCT/ISA/220 (July 2014)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below
RIC/FP7230618		
International application No	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP2016/081430	16 December 2016 (16-12-2016)	16 December 2015 (16-12-2015)
Applicant		
SINGAPORE HEALTH SERVICES PTE LTD		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18 A copy is being transmitted to the International Bureau

This international search report consists of a total of 5 sheets

[X] It is also accompanied by a copy of each prior art document cited in this report

1 **Basis of the report**

a With regard to the **language,** the international search was carried out on the basis of

[X] the international application in the language in which it was filed

[ ] a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12 3(a) and 23.1(b))

b [ ] This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43 6*bis*(a))

c [X] With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No I.

2 [ ] **Certain claims were found unsearchable** (See Box No II)

3 [ ] **Unity of invention is lacking** (see Box No III)

4 With regard to the **title,**

[X] the text is approved as submitted by the applicant

[ ] the text has been established by this Authority to read as follows

5 With regard to the **abstract,**

[X] the text is approved as submitted by the applicant

[ ] the text has been established, according to Rule 38 2, by this Authority as it appears in Box No IV The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

6 With regard to the **drawings,**

a the figure of the **drawings** to be published with the abstract is Figure No 1

[X] as suggested by the applicant

[ ] as selected by this Authority, because the applicant failed to suggest a figure

[ ] as selected by this Authority, because this figure better characterizes the invention

b [ ] none of the figures is to be published with the abstract

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/081430

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

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

a. [X] forming part of the international application as filed

[X] in the form of an Annex C/ST 25 text file

[ ] on paper or in the form of an image file

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

c [ ] furnished subsequent to the international filing date for the purposes of international search only

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

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

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

3 Additional comments

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/081430

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/24 C07K16/28 A61K39/00
ADD.

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 C12N A61K

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)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	M. OBANA ET AL: "Therapeutic Activation of Signal Transducer and Activator of Transcription 3 by Interleukin-11 Ameliorates Cardiac Fibrosis After Myocardial Infarction", CIRCULATION, vol. 121, no. 5, 9 February 2010 (2010-02-09), pages 684-691, XP055359357, ISSN: 0009-7322, DOI: 10.1161/CIRCULATIONAHA.109.893677 page 685, left-hand column, paragraphs 1-3,5 page 686, right-hand column, paragraph 1 - page 687, right-hand column, paragraph 2; figure 2 page 689, left-hand column, paragraph 1 - page 690, right-hand column, paragraph 2 ----- -/--	1-34

[X] Further documents are listed in the continuation of Box C

[X] 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
7 April 2017	18/04/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P B 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel (+31-70) 340-2040, Fax (+31-70) 340-3016	Page, Michael

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/081430

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	M Stangou ET AL: "Effect of IL-11 on glomerular expression of TGF-beta and extracellular matrix in nephrotoxic nephritis in Wistar Kyoto rats", J Nephrol, 1 January 2011 (2011-01-01), pages 106-111, XP055359363, Retrieved from the Internet: URL:http://www.ncbi.nlm.nih.gov/pubmed/20640990 [retrieved on 2017-03-28] abstract page 106, right-hand column, paragraph 1-3 page 107, right-hand column, paragraph 4 - page 108, right-hand column, paragraph 1 page 109, left-hand column, paragraph 1 - right-hand column, paragraph 1 page 110, left-hand column, paragraph 2	1-34
A	US 2010/093976 A1 (AZUMA JUNICHI [JP] ET AL) 15 April 2010 (2010-04-15) paragraphs [0001], [0019], [0086], [0087]; claims 8,9; figure 10	1-34
A	AHROM HAM ET AL: "Critical Role of Interleukin-11 in Isoflurane-mediated Protection against Ischemic Acute Kidney Injury in Mice", ANESTHESIOLOGY., vol. 119, no. 6, 1 December 2013 (2013-12-01), pages 1389-1401, XP055359340, PHILADELPHIA, PA, US ISSN: 0003-3022, DOI: 10.1097/ALN.0b013e3182a950da page 7, paragraph 4 - page 8, paragraph 5	1-34
X	WO 98/36061 A2 (UNIV MANCHESTER [GB]; FERGUSON MARK WILLIAM JAMES [GB]; KANE SHARON O) 20 August 1998 (1998-08-20) page 6, paragraph 2 - page 11, paragraph 1; claims 1,5	1-34
X	WO 00/78336 A1 (GENETICS INST [US]; UNIV JOHNS HOPKINS [US]) 28 December 2000 (2000-12-28) page 1, lines 13,14 page 5, line 27 - page 6, line 32 page 15, lines 19-23; claims 13,14,17,21,22	1-34

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2016/081430

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2010093976 A1	15-04-2010	EP 2077116 A1	08-07-2009
		JP 5191393 B2	08-05-2013
		US 2010093976 A1	15-04-2010
		WO 2008050789 A1	02-05-2008
WO 9836061 A2	20-08-1998	AU 6220398 A	08-09-1998
		WO 9836061 A2	20-08-1998
WO 0078336 A1	28-12-2000	AU 5756100 A	09-01-2001
		WO 0078336 A1	28-12-2000

Information on Search Strategy - Pilot phase (see OJ 2015, A86)
The type of information contained in this sheet may change during the pilot for improving the usefulness of this new service

Application Number
PCT/EP2016/081430

TITLE: TREATMENT OF FIBROSIS

APPLICANT: SINGAPORE HEALTH SERVICES PTE LTD

IPC CLASSIFICATION: C07K16/24, C07K16/28, A61K39/00

EXAMINER: Page, Michael

CONSULTED DATABASES: NPL, WPI

CLASSIFICATION SYMBOLS DEFINING EXTENT OF THE SEARCH:

IPC:

CPC: C07K16/244, C07K16/2866, C07K2317/24, C07K2317/76, C07K2319/00, C12N2310/14, A61K2039/505, C07K2317/21, C07K2317/73

FI/F-TERMS:

KEYWORDS OR OTHER ELEMENTS FEATURING THE INVENTION:
Interleukin 11, IL-11, Interleukin 11 receptor, IL-11R, fibrosis, fibrogenesis, fibrotic

EPO FORM P04A42

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2016/081430	16.12.2016	16.12.2015

International Patent Classification (IPC) or both national classification and IPC
INV. C07K16/24 C07K16/28 A61K39/00

Applicant
SINGAPORE HEALTH SERVICES PTE LTD

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA	Date of completion of this opinion	Authorized Officer
European Patent Office D-80298 Munich Tel +49 89 2399 - 0 Fax +49 89 2399 - 4465	see form PCT/ISA/210	Page, Michael Telephone No +49 89 2399-0

Form PCT/ISA/237 (Cover Sheet) (January 2015)

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

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

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

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

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

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

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

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

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

5. Additional comments:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-34
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-34
Industrial applicability (IA)	Yes:	Claims	1-34
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

1 **Re Item V**

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1.1 **Reference is made to the following documents:**

D1 M. OBANA ET AL: "Therapeutic Activation of Signal Transducer and Activator of Transcription 3 by Interleukin-11 Ameliorates Cardiac Fibrosis After Myocardial Infarction",
CIRCULATION,
vol. 121, no. 5, 9 February 2010 (2010-02-09), pages 684-691, XP055359357,
ISSN: 0009-7322, DOI: 10.1161/CIRCULATIONAHA.109.893677

D2 M Stangou ET AL: "Effect of IL-11 on glomerular expression of TGF-beta and extracellular matrix in nephrotoxic nephritis in Wistar Kyoto rats",
J Nephrol, 1 January 2011 (2011-01-01), pages 106-111, XP055359363,
Retrieved from the Internet:
URL:http://www.ncbi.nlm.nih.gov/pubmed/20640990
[retrieved on 2017-03-28]

D3 US 2010/093976 A1 (AZUMA JUNICHI [JP] ET AL) 15 April 2010 (2010-04-15)

D4 AHROM HAM ET AL: "Critical Role of Interleukin-11 in Isoflurane-mediated Protection against Ischemic Acute Kidney Injury in Mice",
ANESTHESIOLOGY.,
vol. 119, no. 6, 1 December 2013 (2013-12-01), pages 1389-1401, XP055359340,
PHILADELPHIA, PA, US
ISSN: 0003-3022, DOI: 10.1097/ALN.0b013e3182a950da

D5 WO 98/36061 A2 (UNIV MANCHESTER [GB]; FERGUSON MARK WILLIAM JAMES [GB]; KANE SHARON O) 20 August 1998 (1998-08-20)

D6 WO 00/78336 A1 (GENETICS INST [US]; UNIV JOHNS HOPKINS [US]) 28 December 2000 (2000-12-28)

1.2 **Novelty - Art.33(1) and (2) PCT:**

Antagonists of IL-6 and IL-13 for preventing fibrosis are known in the art (D5, D6). Not, however, IL-11.

1.3 **Inventive Step - Art.33(1) and (3) PCT:**

D5 and D6 are both considered to be equivalent closest prior art in that they solve the same technical problem; prevention of fibrosis through antagonism of pro-fibrotic interleukins (claims 1-23), determination of patients suitable for treatment with the same (claims 24 and 25) diagnosis (claims 26-28, 31 and 32) or prognosis (claims 29 and 30).

The subject matter of claims 1-34 is not regarded as being inventive as it appears that the technical problem has not been solved.

The results and conclusions of D1-D4 all call into question the conclusions drawn by the Applicant that antagonists of IL-11 have the claimed technical effect. D1 and D2 both teach that IL-11 prevents fibrosis in heart tissue. D3 teaches the same for the kidney.

Although the application demonstrates that IL-11 is upregulated in fibroblasts in response to TGFβ1 (as expected; Example 2), this is considered to correspond to the activation state of fibroblasts. *In vivo* experiments demonstrated a correlation to collagen production in kidney, heart and liver tissues (Figure 8).

Example 5 documents that IL-11 is pro-fibrotic in a number of *in vitro* assays involving cultured cells.

Given that D1, D2 and D3 all comprise data from animal experiments, whereas the data in the examples is largely *in vitro* from cell culture, the Examiner takes the view that on balance, it is the results of the application which are artificial and the claimed thereapeutic and diagnostic applications are not considered to be viable.

2 **Re Item VIII**

Certain observations on the international application

2.1 Claim 1 of the application recites an agent capable of inhibiting the action of IL-11 for use in a method of treating or preventing fibrosis. Claim 3 concerns a method of treating or preventing fibrosis using suchan agent.

The prevailing opinion in both the patent- and in the scientific literature is that IL-11 is an anti-fibrotic agent (D1, D2, D3). This stands in direct contradiction to the results of Examples 2, 3, 5 and 7 of the application.

As stated above, D1 and D2 both teach that IL-11 prevents fibrosis in heart tissue. D3 focusses on the kidney.

The application demonstrates that IL-11 is upregulated in fibroblasts in response to TGFβ1 (as expected; Example 2) but also that this corresponds to the activation state of fibroblasts. In vivo experiments demonstrated a correlation to collagen production in kidney, heart and liver tissues (Figure 8).

Example 5 documents that IL-11 is pro-fibrotic in a number of *in vitro* assays involving cultured cells.

Given that D1, D2 and D3 all comprise data from animal experiments, whereas the data in the examples is largely *in vitro* from cell culture, the Examiner takes the view that on balance, it is the results of the application which are artificial and the claimed thereapeutic and diagnostic applications are not considered to be supported by the description. Consequently, none of claims 1-34 are considered to meet the support requirements of Article 6 PCT since neither diagnosis nor treatment are plausibly achieved.

2.2 Claims 1-6, 9, 10 and 12-23 are all unclear insofar as the skilled person is left in the dark with respect to the structural nature of the agent (Article 6 PCT).

2.3 Claims 4-11 and 15-23 are all mixed category claims and as such are not considered to meet the clarity requirements of Article 6 PCT. In order to be clear, claims should pertain to a method or a product, but not both.

2.4 No unified criteria exist in the PCT Contracting States on the question whether methods of treatment are industrially applicable, as they are not considered to be industrially applicable in the EPC. No opinion can be given, therefore, on the industrial applicability of claims 3-11 and 14-23, which all claim such methods.

Possible steps after receipt of the international search report (ISR) and written opinion of the International Searching Authority (WO/ISA)

General information

For all international applications, the competent International Searching Authority (**ISA**) will establish an international search report (**ISR**) accompanied by a written opinion of the International Searching Authority (**WO/ISA**). The **WO/ISA** may be responded to by

- filing informal comments with the **International Bureau of WIPO (IB)** (where no demand for international preliminary examination (**demand**) is filed)
- filing amendments under Art. 19 PCT (this can be done whether or not a **demand** is filed)
- filing amendments under Art. 34 PCT and/or formal observations in response to objections raised in the **WO/ISA** (where a **demand** *is* actually filed)

This document explains these possibilities.

Filing informal comments

After receipt of the **ISR and WO/ISA**, the applicant may file informal comments on the **WO/ISA, directly with the IB** (see International Search and Preliminary Examination Guidelines 2.15). These will be communicated to the designated/elected Offices, together with the International Preliminary Report on Patentability (**IPRP**) at 30 months from the priority date.

Amending claims under Art. 19 PCT

The applicant may file **amended claims** under Art. 19 PCT, **directly with the IB** by the later of the following dates:

- 2 months from the date of mailing of the **ISR** and the **WO/ISA**
- 16 months from the priority date

However, any such amendment received by the **IB** after the expiration of the applicable time limit shall be **considered to have been received on time** by the **IB**, if it reaches it **before** the technical preparations for international publication have been completed (the 15th day prior to the date of publication, see PCT Applicant's Guide, International Phase, 9.013).

For further information, please see Rule 46 PCT as well as form PCT/ISA/220.

Please also note that, when filing amended claims under Art. 19 PCT, such amendments shall be **accompanied by a letter** identifying the amendments made and also the basis for the amendments in the application as originally filed (Rule 46.5(b) PCT). Where a **demand** is filed, failure to comply with this requirement may result in the amendments being ignored in the International Preliminary Examination Report (**IPER**), see Rule 70.2(c-*bis*) PCT.

Filing a demand for international preliminary examination

In principle, the **WO/ISA** will be considered to be the written opinion of the International Preliminary Examining Authority (**IPEA**). Where the **WO/ISA** issued by the **EPO** as **ISA** gives a positive opinion on the international application and the invention to which it relates, filing a **demand** with the **EPO** as **IPEA** would normally be unnecessary, since a positive IPRP would anyway be established by the IB based on the WO/ISA (see also further below).

If the applicant wishes to file a **demand** (for example, to allow him to argue his case in international preliminary examination with regard to objections raised in a negative **WO/ISA** before the **IPEA** issues an **IPER**), this must be done before expiration of **3 months after the date of mailing of the ISR and WO/ISA** or **22 months after priority date,** whichever expires later (Rule 54*bis* PCT). Amendments under Art. 34 PCT can be filed with the IPEA, normally at the same time as filing the demand (Rule 66.1(b) PCT) or within the time limit set for reply to any written opinion issued during international preliminary examination by the **IPEA**.

If a **demand** is filed at the **EPO** as **IPEA** and no comments/amendments have been received by the time the **EPO** starts drawing up the **IPER** (Rule 66.4*bis* PCT), the **WO/ISA** will be transformed by the **IPEA** into an **IPER** (also called the **IPRP (Chapter II)** which would merely reflect the content of the **WO/ISA** (OJ 10/2011, 532). The **demand** can still be withdrawn (Art. 37 PCT).

Please also note that, when filing amendments under Art. 34 PCT, such amendments shall be accompanied by a letter which identifies the amendments made and also the basis for the amendments in the application as originally filed (Rule 66.8(a) PCT). Failure to comply with this requirement may result in the amendments being ignored in the **IPER (IPRP (Chapter II))**, see Rule 70.2(c-*bis*) PCT.

Filing a request for supplementary international search

The applicant may, with the **IB**, file a request for **supplementary international search** under Rule 45*bis*.1 PCT. The present **ISR** and **WO/ISA** may also be taken into account in the execution of that supplementary international search, provided that these are available to the Authority charged with this task before it starts the supplementary search (Rule 45*bis*.5 PCT).

This kind of request **cannot be filed specifying the ISA** who did the **international search**.

More information on this topic can be found in the **PCT Applicant's Guide**, Chapter 8 (http://www.wipo.int/pct/en/guide/ip08.html).

End of the international phase

Where no **demand** is filed, at the end of the international phase, the **IB** will transform the **WO/ISA** into the IPRP (PCT Chapter I) (Rule 44*bis* PCT), which will then be transmitted together with possible informal comments to the designated Offices. Where a demand is filed, the **WO/ISA** is not transformed into an **IPRP (Chapter I)** by the **IB**, but rather the **IPEA** will establish an **IPER**, (the IPER is the same as the IPRP (PCT Chapter II), see Rule 70.15 PCT).

BNSDOCID <XS____2006100103CF_I_>

MEWBURN ELLIS INTELLECTUAL PROPERTY
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European Patent Office
D-80298 München
Germany

14 August 2017

FILED ELECTRONICALLY

BEFORE THE EUROPEAN PATENT OFFICE (EPO)
ACTING AS THE
INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY (IPEA)

Dear Sirs

International Patent Application No. PCT/EP2016/081430
Applicant: SINGAPORE HEALTH SERVICES PTE LTD et al.
Our Ref: APG/FP7230618

This letter accompanies the Chapter II Demand filed in connection with this application.

1. Fees

The following fees are being paid online today:

PCT Preliminary Examination Fee	Euro	1930
PCT Handling Fee	Euro	183
TOTAL:	Euro	2113

If any additional fees are required so that this Chapter II Demand is deemed to be filed, then the EPO is authorised to deduct such fees from my firm's deposit account number 2805.0013 under the reference SHORTFALL, informing me in writing that this has been done.

2. Amendments

Amended claims 1 to 14 are filed under Article 34 PCT on replacement pages 94 and 95. For the examiner's convenience a marked up version is included for reference only.

Independent claims 1, 2 and 3 are amended to specify that the agent is an antibody capable of binding to Interleukin 11 (IL-11) or IL-11 receptor α (IL-11Rα), and is capable of inhibiting IL-11 mediated signalling. Basis for the amendment comes from, e.g. page 4, lines 4-13; page 9, line 33; and page 10, lines 19 to 22 of PCT/EP2016/081430 as filed.

Claims 1, 2 and 3 are also amended to specify use treating/preventing fibrosis in a human. Basis comes from e.g. page 42, lines 2-5.

Claim 5 is amended for conformity with claims 1, 2 and 3 as currently amended.

Previous claims 6 to 8 are deleted.

Claim 6 (previous claim 9) is amended for conformity with claims 1, 2 and 3 as currently amended, and also to specify IL-11Rg. Basis comes from, e.g. page 9, line 33.

Previous claims 10 to 15 are deleted.

Claims 7 to 14 (previous claims 16 to 23) are amended editorially for improved readability, and for conformity with amendments to the claims from which they depend.

Previous claims 24 to 34 are deleted.

It is hereby submitted that the claim amendments do not extend beyond the disclosure of the international application as filed, in accordance with Article 34(2)(b) PCT, second sentence.

3. Novelty

I thank the Examiner for recognising that the use of antagonists of IL-11 for preventing fibrosis is not known in the art, and for acknowledging the novelty for the claims.

4. Inventive Step

The Examiner comments that the subject-matter of the claims is not considered to be inventive as the technical problem of treating/preventing fibrosis is not solved.

The Examiner considers that the prior art shows that IL-11 prevents fibrosis in heart and kidney tissue, and so an agent capable of inhibiting the action of IL-11 would not be useful to treat/prevent fibrosis.

Favourable reconsideration of the objection is respectfully requrested in view of the following submissions.

4.1 Prior art studies relating to the role of IL-11

Obana et al., Circulation (2010) 121(5):684-691 (**D1**) is cited by the Examiner as evidence that IL-11 prevents fibrosis in heart tissue. **D1** discloses that treatment with IL-11 reduced the fibrosis area 14 days after myocardial infarction (MI) in a mouse model of MI by coronary ligation (see e.g. Abstract).

Importantly, in the experiments reported in **D1** mice were treated with recombinant human IL-11 – see page 685, left column, paragraph entitled 'Coronary Artery Ligation and IL-11 Treatment', final sentence:

> *"In the IL-11 group, recombinant **human** IL-11 (Peprotech) was administered intravenously for 5 days consecutively; the control group received the same volume of phosphate-buffered saline (PBS) during the same period."* [emphasis added]

Stangou et al., J Nephrol (2011) 106-111 (**D2**) is also cited by the Examiner. This document discloses suppression of glomerular expression of TGFβ1 and extracellular matrix deposition in rat models of glomerulonephritis following administration of a high dose of recombinant human IL-11 (i.e. rhIL-11; see e.g. page 106, right column, second sentence).

The Abstract to **D2**, 'Methods; and 'Results' provide:

> *"Methods: Following induction of nephrotoxic nephritis, expression of TGF-β1, α-smooth muscle actin (α-SMA), fibronectin and p-p38 MAPK was detected in the kidney. **Rats were treated** either with vehicle or **rhIL-11** at a high or low dose and culled on day 6.*
> *Results: A high dose of **rhIL-11** resulted in a significant reduction in the glomerular expression of TGF-β1 (0.4 ± 0.1 vs. 2.04 ± 0.4 semiquantitative score, p<0.005), α-SMA (0.6 ± 0.2 vs. 1.5 ± 0.3, p<0.01) and fibronectin (0.6 ± 0.1 vs. 1.5 ± 0.1, p<0.02). The periglomerular expression of α-SMA and fibronectin was significantly reduced in rats treated with the high dose of **rhIL-11** (9.6% ± 2% vs. 92% ± 2.5% of glomeruli, p<0.01; and 26% ± 4.9% vs. 94% ± 1.9% of glomeruli, p<0.005, respectively). There was a slight but insignificant reduction of p-p38 MAPK in IL-11 treated rats. Treatment with low-dose rhIL-11 did not reduce expression of these molecules."*
> [emphasis added]

US 2010/093976 A1 (**D3**) cited by the Examiner is the publication of a US patent application from the research group of **D1** (led by Prof. Yasushi Fujio at Osaka University). Paragraphs [0085]-[0087] disclose that in a myocardial infarction model, mice treated with IL-11 had a reduced infarct area (measured as the proportion of fibrotic area in the left ventricular myocardium), as compared to PBS-treated mice.

Whilst the species of IL-11 used in **D3** is not disclosed, it appears highly likely that this is human IL-11. Paragraph [0058] discloses that the IL-11 was obtained from PeproTech, which is the same source as the recombinant human IL-11 used by the same researchers for the experiments of **D1**. Furthermore, Kimura et al., Cytokine (2007) 38(2):107-15 (Kimura; enclsoed), is another publication by the same research group which describes many of the experiments and results disclosed in **D3** – compare Figs. 2-4 of **D3** with Figs. 3a-c of Kimura; Fig. 5 of **D3** with Fig. 6 of Kimura; and Fig. 9 of **D3** with Fig. 1 of Kimura.

Page 108, left column, last complete paragraph of Kimura makes clear that the experiments used recombinant human IL-11, which was again purchased from Peprotech.

Therefore, each of **D1** to **D3** report the results of studies which are fundamentally flawed in their design, for seeking to analyse the role of IL-11 in pathophysiological processes by analysing effects following administration of the human IL-11 molecule in rodent models of disease processes.

The effects observed in these studies following administration of human IL-11 cannot be separated from, for example, the mouse/rat host response to this non-host molecule.

Furthermore, it cannot be assumed that effects observed for human IL-11 in mouse and rat would accurately reflect the role of human IL-11 in fibrosis in humans. Human IL-11, IL11-Rα and gp130 molecules have distinct amino acid sequences to their mouse and rat homologues, and so it cannot be assumed that human IL-11 would interact with mouse and rat receptor molecules to effect the same outcomes as would be observed in humans.

This is further supported by Figure 20F of the present application, which shows that mouse fibroblasts are considerably more sensitive to activation *in vitro* to a pro-fibrotic myofibroblast phenotype by treatment with murine IL-11 as compared to treatment with human IL-11 (see accompanying legend on page 55).

Ham et al., Anesthesiology (2013) 119(6): 1389-1401 (**D4**) is also cited by the Examiner. This document reports induction of IL-11 mRNA and protein expression by human kidney proximal tubule cells and mouse kidney cells cultured *in vitro*, and by mouse kidney *in vivo*, following treatment with isofluorane.

Figures 6-9 are disclosed to show protective effects for IL-11 following ischemic acute kidney injury, using IL-11R WT and knockout mice. We note that these assays evaluate correlates of renal function, necrosis, neutrophil infiltration and apoptosis. None of the experiments of **D4** investigate the effect of IL-11 in fibrosis.

It will be clear from the foregoing explanation that it is not possible to draw any scientifically meaningful conclusions as to the role of IL-11 in fibrosis from **D1** to **D4**.

Moreover, **Annex I** filed herewith provides further evidence that the experiments performed in **D1** are invalid, and that conclusions drawn from the results are fallacious.

Annex I reports the results of studies in which myocardial infarct was generated in mice by coronary artery ligation, or sham-operated, and wherein the mice were then treated either with PBS or with recombinant mouse IL-11.

Histological analysis of heart tissue sections revealed an increase in several markers of the fibrosis associated with IL-11 treatment (Figure 1). Figure 1A of **Annex I** shows increased collagen deposition (a known indicator of fibrosis) as determined by Masson's trichrome staining. Figure 1A also reveals upregulated expression of αSMA (as measured by ACTA2 staining) – a marker of fibroblast activation. Activated fibroblasts are known to be the central effectors of the fibrotic response post-myocardial infarction, and so the level of ACTA2 is an extremely reliable indicator of the fibrotic response. Figures 1B and 1C reveal that IL-11 treatment was associated with a statistically significant increase in epicardial thickness at the border of the infarct and at the right ventricle, whilst Figures 1D and 1E show IL-11 treatment caused a statistically significant reduction in heart function, as determined by analysis of the ejection fraction and fractional shortening.

Annex I indicates that treatment of mice with mouse IL-11 following myocardial infarction increases fibrosis, and is associated with a reduction in heart function.

4.2 The data of the present application

The present application provides a wealth of data demonstrating a pro-fibrotic role for IL-11, and establishing the therapeutic utility of antagonists of IL-11 mediated signalling in the treatment/prevention of fibrosis.

Some of the key data are highlighted below.

Example 2 at page 60, third paragraph and Figures 7A and 7B demonstrate that incubation of primary human atrial fibroblasts with recombinant human IL-11 increases deposition of collagen by fibroblasts, a well-established fibrotic process. Moreover, treatment with neutralising anti-IL-11 antibody (but not isotype control antibody) is shown to abrogate

collagen production induced by stimulation of the fibroblasts with TGFβ1 (a known pro-fibrotic stimulus).

Example 3 at page 61, and Figure 10 further demonstrate the ability of neutralising anti-IL-11 antibody to abrogate increased collagen production by human atrial fibroblasts in response to various other pro-fibrotic stimuli (ANG2, PDGF, ET-1).

Example 5.2 at pages 63-64 and Figures 20A-20E provide further data supporting a pro-fibrotic role for IL-11 in heart tissue. Human atrial fibroblasts were shown to display significantly increased production of extracellular matrix components (collagen, periostin) and increased expression of pro-fibrotic markers (αSMA, IL-6, MMP2, TIMP1) in response to treatment with human IL-11 protein, in the same way as production of these factors is increased in response to treatment with the pro-fibrotic stimulus TGFβ1.

Example 5.3.1 and Figures 38A to 38D likewise show increased production of extracellular matrix components and increased expression of fibrotic markers by human primary liver fibroblasts in response to treatment with human IL-11, and also the ability of neutralising anti-IL-11 antibody to abrogate the profibrotic effects of stimulation with TGFβ1.

Figures 22A to 22F and 23A and 23B show that TGFβ1-mediated fibrosis can be inhibited by treatment with neutralising anti-IL-11 antibody. Figure 24 moreover shows that IL-11-binding decoy receptor molecules, neutralising anti-IL-11Rα antibodies and oligonucleotides encoding siRNA for antisense knockdown of *IL-11* and *IL-11RA* gene expression are similarly able to inhibit TGFβ1-mediated transition of fibroblasts to myofibroblasts (fibrosis effector cells). Further data showing inhibition of the TGFβ1-mediated fibrotic response using decoy IL-11 receptors is provided at Figures 32A and 32B.

Example 5.3.3 at page 64-65 and Figure 21B and 21C provide *in vivo* data demonstrating IL-11 to be pro-fibrotic in a variety of tissues. Injection of mice with recombinant mouse IL-11 caused an increase in the relative weight of heart, kidney, lung and liver (Figure 21B), and that this was associated with increased collagen content in these tissues (Figure 21C).

Further *in vivo* data supporting a pro-fibrotic role for IL-11 is provided at Examples 7.2 and 7.3, and Figures 27A to 27D and Figure 28. These experiments show that IL-11RA knockout mice are protected from fibrosis of the heart and kidney tissues induced by profibrotic stimuli, indicating signalling through the IL-11 receptor as an important mediator of fibrotic processes. Further still, Figures 31A and 31B, summarised at the legend to Figure 31 on page 57 – more fibrosis was detected in eye sections obtained from wildtype mice than IL-11RA knockout mice at 7 days following trabeculectomy.

Thus the present application provides abundant data from both *in vitro* and *in vivo* studies proving that IL-11/IL-11R signalling is a key mediator of fibrosis in a wide range of tissues, and demonstrates that inhibition of IL-11 mediated signalling reduces fibrosis, as determined by analysis of a variety of markers of the fibrotic response.

4.3 Inventive step

At section 1.3 of the WO/ISA the Examiner identifies **D5** and **D6** as being equivalent closest prior art to the present invention, each relating to prevention of fibrosis through antagonism of pro-fibrotic interleukins.

Neither of **D5** nor **D6** disclose treatment of fibrosis using an antibody which is capable of binding to IL-11 or IL-11Rα and inhibiting IL-11 mediated signalling (cf. independent claims 1, 2 and 3).

Starting from either of **D5** or **D6**, the technical problem to be solved by the present invention can be formulated as "the provision of an alternative treatment for the prevention or treatment of fibrosis".

The claimed solution to use an agent which is capable of binding to IL-11 or IL-11Rα and inhibiting IL-11 mediated signalling would not have been obvious to the skilled person at the relevant date.

As explained in the present application e.g. at page 1, line 31 to page 2 line 24, the role of IL-11 in fibrosis was not clear at the priority date for the present application, and the majority of studies suggested that IL-11 is anti-fibrotic, as evidenced e.g. by **D1** to **D3** identified by the Examiner.

In view of the state of the art at the relevant date, the skilled person simply would not have arrived at the subject-matter of the present independent claims. In view of the uncertainty as the role of IL-11 in fibrosis, the skilled person would not have considered to use antagonists of IL-11 mediated signalling to treat/prevent fibrosis with a reasonable expectation of success.

Rather, only the extensive data of the present application from *in vitro* and *in vivo* studies described under section 4.2 above make plausible the use of antagonists of IL-11 mediated signalling for the treatment or prevention of fibrosis.

Accordingly, the subject-matter of independent claims 1 to 3, and therefore the claims dependent therefrom, involve an inventive step over the cited prior art documents (Article 33(3) PCT).

5. Support

At section 2.1 of the WO/ISA the Examiner objects that the claims lack support, as evidenced by **D1** to **D3**.

Favourable reconsideration of the objection is respectfully requested in view of sections 4.1 and 4.2 above, which explain failings of the prior art studies in relation to the role of IL-11 in fibrosis, and which make clear that the present application supports the therapeutic utility of anti-IL-11 and anti-IL-11Rα antibody antagonists of IL-11 mediated signalling to treat/prevent fibrosis.

6. Clarity

6.1 Structural nature of the agent

The Examiner objects at section 2.2 of the WO/ISA that the claims lack clarity in respect of the structural nature of the agent used to inhibit the action of IL-11.

The independent claims are currently amended to specify an antibody which is capable of binding to IL-11 or IL-11Rα and inhibiting IL-11 mediated signalling.

As explained in section 4.2 above, the application demonstrates the use of anti-IL-11 antibodies and anti-IL-11Rα antibodies to bind to IL-11/IL-11Rα and inhibit IL-11 mediated signalling (see e.g. Figure 24).

The skilled person is able to determine whether a given anti-IL-11 antibody or anti-IL-11Rα is capable of inhibiting IL-11 mediated signalling with certainty and without undue burden.

6.2 Claims depending from claims in different categories

At section 2.3 of the WO/ISA the Examiner objects that certain dependent claims lack clarity for depending from claims in different categories.

Favourable reconsideration of the objection is respectfully requested in view of the present amendments to the claims and the following comments.

The objection pertains to claims which depend from independent claims relating to methods of treatment and medical uses, drafted in accordance with the different practices of national/regional patent offices relating to the allowability of claims defining methods of treatment, and allowable medical use claim formats.

The dependent claims specify further features of the claim in the appropriate format, as is clear from the preamble to the claims. There is no lack of clarity.

Take the example of present claim 4:

> *"4. The antibody for use in a method of treating or preventing fibrosis according to claim 1, the use according to claim 2, or the method according to claim 3, wherein the antibody is capable of preventing or reducing the binding of IL-11 to an IL-11 receptor."*

This claim clearly specifies further features of the antibody specified in respective medical use and method of treatment claims, and the skilled person is left with no uncertainty in this respect.

All claims satisfy the requirements of Article 6 PCT.

7. Industrial applicability – methods of treatment

At section 2.4 the Examiner comments that no opinion can be given in respect of the industrial applicability of claims relating to methods of treatment.

The applicant intends to address this issue as necessary before the different offices during the national/regional phase.

8. Closing Remarks

It is requested that an International Preliminary Report on Patentability (IPRP/Chapter II) be issued indicating that all claims are novel and inventive.

In the event that the IPEA has any outstanding objections we request a telephone interview with the Examiner in accordance with Article 34(2)(a) and Rule 66.6 PCT.

Yours faithfully

Adam Gregory, DPhil
for MEWBURN ELLIS LLP
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Encs. Article 34 Amendments
Copy showing changes
Kimura et al., Cytokine (2007) 38(2):107-15
Annex I

APG/krg

PCT DEMAND FOR INTERNATIONAL PRELIMINARY EXAMINATION (CHAPTER II)

0	For International Preliminary Examining Authority use only	
0-1	Identification of IPEA	
0-2	Date of receipt of DEMAND	

0-3	Form - PCT/IPEA/401 PCT Demand	
0-3-1	Prepared Using	eOLF Version: FMMNGR5166, IBR4015160, FM_FOP0205
0-4	Demand under Article 31 of the Patent Cooperation Treaty: The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).	
0-5	International Preliminary Examining Authority (specified by the applicant)	IPEA/EP
I	Identification of the International application	
I-1	International Application No.	PCT/EP2016/081430
I-2	International Filing Date	16 December 2016 (16.12.2016)
I-3	(Earliest) Priority Date	16 December 2015 (16.12.2015)
I-4	Applicant's or agent's file reference	RIC/FP7230618
I-5	Title of Invention	TREATMENT OF FIBROSIS

PCT DEMAND FOR INTERNATIONAL
PRELIMINARY EXAMINATION (CHAPTER II)

II-1	Applicant	
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II-1-6	Facsimile No.	
II-1-7	e-mail	
II-1-8	Applicant's registration No. with the Office	
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II-2-1	Name	NATIONAL UNIVERSITY OF SINGAPORE
II-2-2	Address	21 Lower Kent Ridge Road 119077 Singapore Singapore
II-2-3	State of nationality	SG
II-2-4	State of residence	SG
II-2-5	Telephone No.	
II-2-6	Facsimile No.	
II-2-7	e-mail	
II-2-8	Applicant's registration No. with the Office	

PCT DEMAND FOR INTERNATIONAL
PRELIMINARY EXAMINATION (CHAPTER II)

II-3	Applicant	
II-3-1	Name (LAST, First)	CLEGG, Richard Ian
II-3-2	Address	City Tower 40 Basinghall Street London Greater London EC2V 5DE United Kingdom
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II-3-4	State of residence	GB
II-3-5	Telephone No.	
II-3-6	Facsimile No.	
II-3-7	e-mail	
II-3-8	Applicant's registration No. with the Office	

PCT DEMAND FOR INTERNATIONAL
PRELIMINARY EXAMINATION (CHAPTER II)

III-1	Agent or common representative; or address for correspondence The person identified below is hereby/ has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	Attorney (appointed earlier)
III-1-1	Name	Adam GREGORY
III-1-1a	Attn., c/o	MEWBURN ELLIS LLP
III-1-2	Address	City Tower 40 Basinghall Street London Greater London EC2V 5DE United Kingdom
III-1-3	Telephone No.	+44 20 7776 5300
III-1-4	Facsimile No.	+44 330 111 4455
III-1-5	e-mail	mail@mewburn.com
III-1-5a	The International Preliminary Examining Authority is authorised to use this e-mail address, if the Authority so wishes, to send notifications issued in respect of this international preliminary examination :	as advanced copies followed by paper notification
III-1-6	Agent's registration No.	
IV	Basis for International Preliminary Examination	
IV-1-1	Statement concerning amendments: The applicant wishes the International Preliminary Examination to start on the basis of:	-the description as originally filed -the sequence listing as originally filed -the claims as amended under Article 34 -the drawings as originally filed
IV-2	Language for the purposes of international preliminary examination:	English -(which is the language in which the international application was filed)
V	Election of States	The filing of this demand constitutes the election of all Contracting States which are designated and are bound by Chapter II of the PCT.

PCT DEMAND FOR INTERNATIONAL
PRELIMINARY EXAMINATION (CHAPTER II)

VI	Check list The demand is accompanied by the following elements, in the language referred to under item IV-2 for the purposes of international preliminary examination:	Number of sheets	Electronic file(s) attached	Received Y/N (For IPEA use only)
VI-2 (1)	Amendments under Art. 34	2	AMDA34-1	
VI-2 (2)	Amendments under Art. 34	5	AMDA34-2	
VI-6	Letter accompanying the amendments under Article 34 (Rule 66.8)	8	LETT.PDF	
VI-7	Other : Annex 1	3	OTHERDOC-1.pdf	
	Accompanying Items The demand is also accompanied by the following item(s):	Details	Electronic file(s) attached	Received Y/N (For IPEA use only)
VI-8	Fee calculation sheet	1	fees.pdf	
VII-1	Signature of applicant, agent or common representative	PKCS7 Digital Signature: Adam Gregory 50163		
VII-1-1	Name	MEWBURN ELLIS LLP		
VII-1-2	Capacity	Representative		
VIII	Note(s) to IPEA/EP			

PCT DEMAND FOR INTERNATIONAL
PRELIMINARY EXAMINATION (CHAPTER II)

FOR INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY USE ONLY

8-1	Date of actual receipt of DEMAND	
8-2	Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b)	
8-3	The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and items 8-4 and 8-5 below do not apply.	
8-3-1	The applicant has been informed accordingly	
8-4	The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.	
8-5	Although the date of receipt of the demand is after the expiration of the 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.	
8-6	The date of receipt of the demand is AFTER the expiration of the time limit under Rule 54bis, 1(a) and item 7 or 8, below, does not apply.	
8-7	The date of receipt of the demand is WITHIN the time limit under Rule 54bis.1(a) as extended by virtue of Rule 80.5.	
8-8	Although the date of receipt of the demand is after the expiration of the time limit under Rule 54bis.1(a), the delay in arrival is EXCUSED pursuant to Rule 82.	

FOR INTERNATIONAL BUREAU USE ONLY

9-1	Demand received from IPEA on	

Claims:

1. An antibody which is capable of binding to Interleukin 11 (IL-11) or IL-11 receptor α (IL-11Rα) and inhibiting IL-11 mediated signalling, for use in a method of treating or preventing fibrosis in a human.

2. Use of an antibody which is capable of binding to Interleukin 11 (IL-11) or IL-11 receptor α (IL-11Rα) and inhibiting IL-11 mediated signalling, in the manufacture of a medicament for use in a method of treating or preventing fibrosis in a human.

3. A method of treating or preventing fibrosis in a human, the method comprising administering to a subject in need of treatment a therapeutically effective amount of an antibody which is capable of binding to Interleukin 11 (IL-11) or IL-11 receptor α (IL-11Rα) and inhibiting IL-11 mediated signalling.

4. The antibody for use in a method of treating or preventing fibrosis according to claim 1, the use according to claim 2, or the method according to claim 3, wherein the antibody is capable of preventing or reducing the binding of IL-11 to an IL-11 receptor.

5. The antibody for use in a method of treating or preventing fibrosis according to claim 1 or 4, the use according to claim 2 or 4, or the method according to claim 3 or 4, wherein the antibody is an IL-11 binding antibody.

6. The antibody for use in a method of treating or preventing fibrosis according to claim 1 or 4, the use according to claim 2 or 4, or the method according to claim 3 or 4, wherein the antibody is an IL-11Rα binding antibody.

7. The antibody for use in a method of treating or preventing fibrosis according to any one of claims 1 or 4 to 6, the use according to any one of claims 2 or 4 to 6, or the method according to any one of claims 3 or 4 to 6, wherein the fibrosis is fibrosis of the heart, liver, kidney or eye.

8. The antibody for use in a method of treating or preventing fibrosis according to any one of claims 1 or 4 to 7, the use according to any one of claims 2 or 4 to 7, or the method according to any one of claims 3 or 4 to 7, wherein the fibrosis is in the heart and

is associated with dysfunction of the musculature or electrical properties of the heart, or thickening of the walls or valves of the heart.

9. The antibody for use in a method of treating or preventing fibrosis according to any one of claims 1 or 4 to 7, the use according to any one of claims 2 or 4 to 7, or the method according to any one of claims 3 or 4 to 7, wherein the fibrosis is in the liver and is associated with chronic liver disease or liver cirrhosis.

10. The antibody for use in a method of treating or preventing fibrosis according to any one of claims 1 or 4 to 7, the use according to any one of claims 2 or 4 to 7, or the method according to any one of claims 3 or 4 to 7, wherein the fibrosis is in the kidney and is associated with chronic kidney disease.

11. The antibody for use in a method of treating or preventing fibrosis according to any one of claims 1 or 4 to 7, the use according to any one of claims 2 or 4 to 7, or the method according to any one of claims 3 or 4 to 7, wherein the fibrosis is in the eye and is retinal fibrosis, epiretinal fibrosis, or subretinal fibrosis.

12. The antibody for use in a method of treating or preventing fibrosis according to any one of claims 1 or 4 to 11, the use according to any one of claims 2 or 4 to 11, or the method according to any one of claims 3 or 4 to 11, wherein the method of treating or preventing comprises administering said antibody to a subject in which IL-11 or IL-11R expression is upregulated.

13. The antibody for use in a method of treating or preventing fibrosis according to any one of claims 1 or 4 to 12, the use according to any one of claims 2 or 4 to 12, or the method according to any one of claims 3 or 4 to 12, wherein the method of treating or preventing comprises administering said antibody to a subject in which IL-11 or IL-11R expression has been determined to be upregulated.

14. The antibody for use in a method of treating or preventing fibrosis according to any one of claims 1 or 4 to 13, the use according to any one of claims 2 or 4 to 13, or the method according to any one of claims 3 or 4 to 13, wherein the method of treating or preventing comprises determining whether IL-11 or IL-11R expression is upregulated in the subject and administering said antibody to a subject in which IL-11 or IL-11R expression is upregulated.

Claims:

1. An ~~agent~~ antibody which is capable of binding to Interleukin 11 (IL-11) or IL-11 receptor α (IL-11Rα) and inhibiting ~~the action of Interleukin-11 (~~IL-11~~)~~ mediated signalling, for use in a method of treating or preventing fibrosis in a human.

2. Use of an ~~agent~~ antibody which is capable of binding to Interleukin 11 (IL-11) or IL-11 receptor α (IL-11Rα) and inhibiting ~~the action of Interleukin-11 (~~IL-11~~)~~ mediated signalling, in the manufacture of a medicament for use in a method of treating or preventing fibrosis in a human.

3. A method of treating or preventing fibrosis in a human, the method comprising administering to a subject in need of treatment a therapeutically effective amount of an ~~agent~~ antibody which is capable of binding to Interleukin 11 (IL-11) or IL-11 receptor α (IL-11Rα) and inhibiting ~~the action of Interleukin-11 (~~IL-11~~)~~ mediated signalling.

4. The ~~agent~~ antibody for use in a method of treating or preventing fibrosis according to claim 1, the use according to claim 2, or the method according to claim 3, wherein the antibody~~agent~~ is ~~an agent~~ capable of preventing or reducing the binding of IL-11 to an IL-11 receptor.

5. The ~~agent~~ antibody for use in a method of treating or preventing fibrosis according to claim 1 or 4, the use according to claim 2 or 4, or the method according to claim 3 or 4, wherein the antibody~~agent~~ is an IL-11 binding ~~agent~~antibody.

~~6. The agent for use in a method of treating or preventing fibrosis, use or method according to claim 5, wherein the IL-11 binding agent is selected from the group consisting of: an antibody, polypeptide, peptide, oligonucleotide, aptamer or small molecule.~~

~~7. The agent for use in a method of treating or preventing fibrosis, use or method according to claim 5, wherein the IL-11 binding agent is an antibody.~~

~~8. The agent for use in a method of treating or preventing fibrosis, use or method according to claim 5, wherein the IL-11 binding agent is a decoy receptor.~~

~~9~~6. The ~~agent~~ antibody for use in a method of treating or preventing fibrosis according to claim 1 or 4, the use according to claim 2 or 4, or the method according to claim 3 or 4, wherein the ~~agent~~ antibody is an ~~IL-11 receptor~~ (IL-11R~~α~~) binding ~~agent~~antibody.

~~10. The agent for use in a method of treating or preventing fibrosis, use or method according to claim 9, wherein the IL-11R binding agent is selected from the group consisting of: an antibody, polypeptide, peptide, oligonucleotide, aptamer or small molecule.~~

~~11. The agent for use in a method of treating or preventing fibrosis, use or method according to claim 9, wherein the IL-11R binding agent is an antibody.~~

~~12. An agent capable of preventing or reducing the expression of interleukin-11 (IL-11) or an interleukin-11 receptor (IL-11R) for use in a method of treating or preventing fibrosis.~~

~~13. Use of an agent capable of preventing or reducing the expression of interleukin-11 (IL-11) or an interleukin-11 receptor (IL-11R) in the manufacture of a medicament for use in a method of treating or preventing fibrosis.~~

~~14. A method of treating or preventing fibrosis, the method comprising administering to a subject in need of treatment a therapeutically effective amount of an agent capable of preventing or reducing the expression of interleukin-11 (IL-11) or an interleukin-11 receptor (IL-11R).~~

~~15. The agent for use in a method of treating or preventing fibrosis according to claim 12, use according to claim 13 or method according to claim 14, wherein the agent is a small molecule or oligonucleotide.~~

~~16~~7. The ~~agent~~ antibody for use in a method of treating or preventing fibrosis according to any one of claims 1 or 4 to 6, the use according to any one of claims 2 or 4 to 6, or the method according to any one of claims 3 or 4 to 6, ~~use or method according to any one of the preceding claims,~~ wherein the fibrosis is fibrosis of the heart, liver, kidney or eye.

~~17~~8. The ~~agent~~ antibody for use in a method of treating or preventing fibrosis according to any one of claims 1 or 4 to 7, the use according to any one of claims 2 or 4 to 7, or the

method according to any one of claims 3 or 4 to 7, ~~use or method according to any one of the preceding claims~~, wherein the fibrosis is in the heart and is associated with dysfunction of the musculature or electrical properties of the heart, or thickening of the walls or valves of the heart.

~~189~~. The ~~agent~~ antibody for use in a method of treating or preventing fibrosis according to any one of claims 1 or 4 to 7, the use according to any one of claims 2 or 4 to 7, or the method according to any one of claims 3 or 4 to 7, ~~use or method according to any one of the preceding claims~~, wherein the fibrosis is in the liver and is associated with chronic liver disease or liver cirrhosis.

~~1910~~. The ~~agent~~ antibody for use in a method of treating or preventing fibrosis according to any one of claims 1 or 4 to 7, the use according to any one of claims 2 or 4 to 7, or the method according to any one of claims 3 or 4 to 7, ~~use or method according to any one of the preceding claims~~, wherein the fibrosis is in the kidney and is associated with chronic kidney disease.

~~2011~~. The ~~agent~~ antibody for use in a method of treating or preventing fibrosis according to any one of claims 1 or 4 to 7, the use according to any one of claims 2 or 4 to 7, or the method according to any one of claims 3 or 4 to 7, ~~use or method according to any one of the preceding claims~~, wherein the fibrosis is in the eye and is retinal fibrosis, epiretinal fibrosis, or subretinal fibrosis.

~~2112~~. The ~~agent~~ antibody for use in a method of treating or preventing fibrosis according to any one of claims 1 or 4 to 11, the use according to any one of claims 2 or 4 to 11, or the method according to any one of claims 3 or 4 to 11, ~~use or method according to any one of the preceding claims~~, wherein the method of treating or preventing comprises administering said ~~agent~~ antibody to a subject in which IL-11 or IL-11R expression is upregulated.

~~2213~~. The ~~agent~~ antibody for use in a method of treating or preventing fibrosis according to any one of claims 1 or 4 to 12, the use according to any one of claims 2 or 4 to 12, or the method according to any one of claims 3 or 4 to 12, ~~use or method according to any one of the preceding claims~~, wherein the method of treating or preventing comprises administering said ~~agent~~ antibody to a subject in which IL-11 or IL-11R expression has been determined to be upregulated.

~~23~~14. The ~~agent~~ antibody for use in a method of treating or preventing fibrosis according to any one of claims 1 or 4 to 13, the use according to any one of claims 2 or 4 to 13, or the method according to any one of claims 3 or 4 to 13, ~~use or method according to any one of the preceding claims~~, wherein the method of treating or preventing comprises determining whether IL-11 or IL-11R expression is upregulated in the subject and administering said ~~agent~~ antibody to a subject in which IL-11 or IL-11R expression is upregulated.

~~24. A method of determining the suitability of a subject for the treatment or prevention of fibrosis with an agent capable of inhibiting the action of Interleukin 11 (IL-11), the method comprising determining, optionally *in vitro*, whether IL-11 or an Interleukin 11 receptor (IL-11R) expression is upregulated in the subject.~~

~~25. A method of selecting a subject for the treatment or prevention of fibrosis with an agent capable of inhibiting the action of Interleukin 11 (IL-11), the method comprising determining, optionally *in vitro*, whether IL-11 or an Interleukin 11 receptor (IL-11R) expression is upregulated in the subject.~~

~~26. A method of diagnosing fibrosis or a risk of developing fibrosis in a subject, the method comprising determining, optionally *in vitro*, the upregulation of Interleukin 11 (IL-11) or an Interleukin 11 receptor (IL-11R) in a sample obtained from the subject.~~

~~27. The method of claim 26, wherein the method is a method of confirming a diagnosis of fibrosis in a subject suspected of having fibrosis.~~

~~28. The method of claim 26 or 27, wherein the method further comprises selecting the subject for treatment with an agent capable of inhibiting the action of IL-11 or with an agent capable of preventing or reducing the expression of IL-11 or IL-11R.~~

~~29. A method of providing a prognosis for a subject having, or suspected of having fibrosis, the method comprising determining, optionally *in vitro*, whether Interleukin 11 (IL-11) or an Interleukin 11 receptor (IL-11R) is upregulated in a sample obtained from the subject and, based on the determination, providing a prognosis for treatment of the subject with an agent capable of inhibiting the action of IL-11 or with an agent capable of preventing or reducing the expression of IL-11 or IL-11R.~~

30. The method of claim 29, wherein the method further comprises selecting a subject determined to have upregulated IL-11 or IL-11R for treatment with an agent capable of inhibiting the action of IL-11 or with an agent capable of preventing or reducing the expression of IL-11 or IL-11R.

31. A method of diagnosing fibrosis or a risk of developing fibrosis in a subject, the method comprising determining, optionally *in vitro*, one or more genetic factors in the subject that are predictive of upregulation of Interleukin-11 (IL-11) or an Interleukin-11 receptor (IL-11R) expression, or of upregulation of IL-11 or IL-11R activity.

32. The method of claim 31, wherein the method is a method of confirming a diagnosis of fibrosis in a subject suspected of having fibrosis.

33. The method of claim 32 or 32, wherein the method further comprises selecting the subject for treatment with an agent capable of inhibiting the action of IL-11 or with an agent capable of preventing or reducing the expression of IL-11 or IL-11R.

34. A method of providing a prognosis for a subject having, or suspected of having, fibrosis, the method comprising determining, optionally *in vitro*, one or more genetic factors in the subject that are predictive of upregulation of Interleukin-11 (IL-11) or an Interleukin-11 receptor (IL-11R) expression, or of upregulation of IL-11 or IL-11R activity.

ANNEX I: Effect of mouse IL-11 in a mouse model of myocardial infarction

Myocardial infarct (MI) was generated in mice by ligation of the coronary artery as described in Obana et al., Circulation (2010) 121(5):684-691. Sham-operated mice were subjected to similar surgery, except that the coronary artery was not ligated.

Beginning at 24 hours after operation, mice were administered subcutaneously either with recombinant mouse IL-11 (100 µg/kg/day), or the same volume of PBS, for 6 consecutive days.

Cardiac function was then analysed by echocardiography as described in Gao et al., Curr Protoc Mouse Biol. (2011) 1:71-83. Ejection fraction (%) was calculated using the formula "EF(%) = 100 x [($LVIDd^3$-$LVIDs^3$)/$LVIDd^3$]", and fractional shortening (%) was calculated using the formula "100 x [(LVIDd-LVIDs)/LVIDd]", where LVIDd and LVIDs = left ventricular internal diameter during diastole and systole, respectively.

Mice were then sacrificed and sections of heart tissue were prepared and analysed by histology for markers of fibrosis.

The results are shown in Figures 1A-1E. Taken together, the results indicate that administration to mice of mouse IL-11 following myocardial infarction increases fibrosis, and is associated with a reduction in heart function.

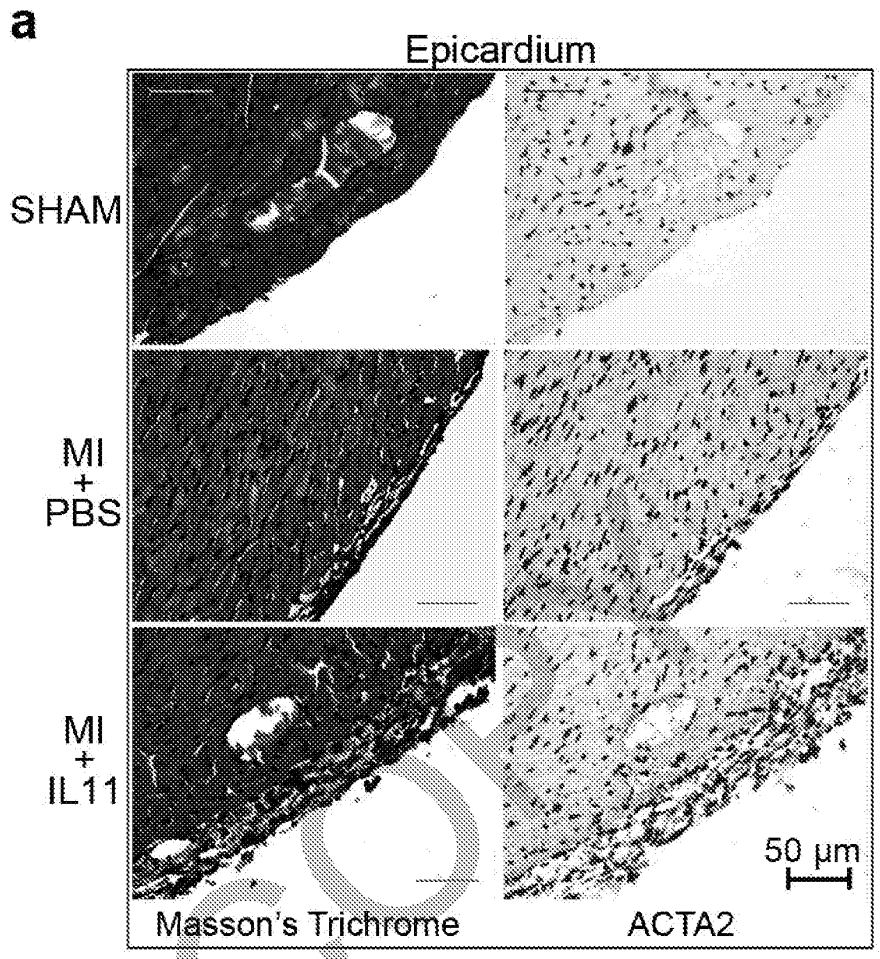

Figure 1A. Representative histological images of the heart after sham operation, myocardial infarct, and treatment with PBS or mouse IL-11. Masson's trichrome revealed increased collagen deposition and fibroblast activation (αSMA, ACTA2 staining) after treatment with mouse IL-11, which was statistically significant in semi-quantitative analysis of tissue sections.

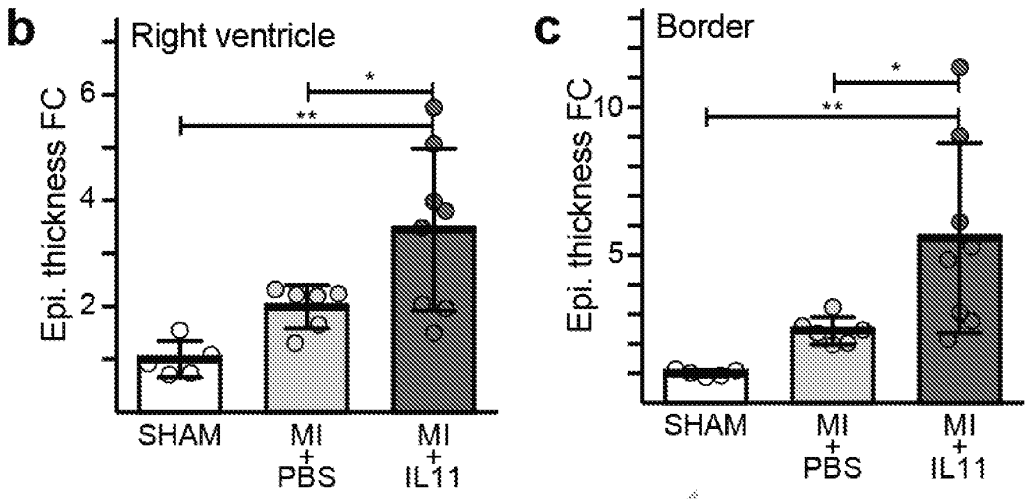

Figure 1B and 1C. Bar charts showing epicardial thickness in (**A**) the right ventricle and (**B**) at the border of the infarct. A statistically significant increase in epicardial thickness was observed at the right ventricle and at the border of the infarct following treatment with mouse IL-11, as compared to sham-operated and PBS-treated mice.

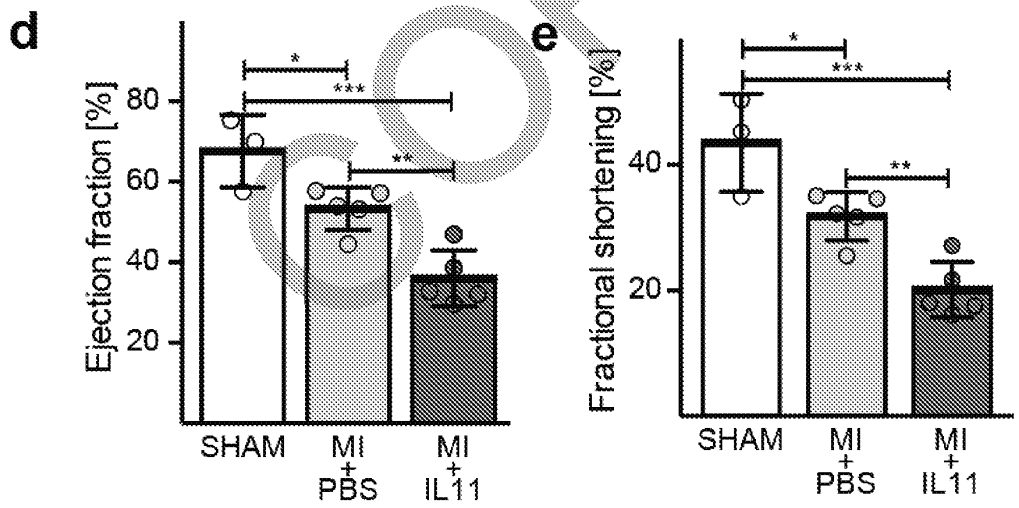

Figure 1D and 1E. Bar charts showing (**A**) ejection fraction and (**B**) fractional shortening. A statistically significant decrease in ejection fraction and fractional shortening was detected, indicating a decrease in heart function following treatment with mouse IL-11, as compared to sham-operated and PBS-treated mice.

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Fee type	Currency	Fee	Total
Fee for preliminary examination of int. application	EUR	1930.00	1930.00
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Total	EUR		2113.00

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Submission number	5533364	
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Documents submitted	package-data.xml fee-sheet.xml pct-demand.pdf (6 p.) validation-log.xml AMDA34-1.PDF (2 p.) LETT.PDF (8 p.)	demand.xml fees.pdf (1 p.) ValidLog.pdf (1 p.) olf-specific-data.xml AMDA34-2.PDF (5 p.) OTHERDOC-1.PDF (3 p.)
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ADA, Supplement to OJ EPO 3/2014 (see decision T 152/82, OJ EPO 1984, 301).

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Singapore Health Services PTE LTD et al.	Confirmation No.:	2829
Application No.:	15/381,622	Art Unit:	1646
Filing Date:	December 16, 2016	Examiner:	Prema Maria Mertz
Title:	TREATMENT OF FIBROSIS		

THIRD PARTY PREISSUANCE SUBMISSION UNDER 37 C.F.R. § 1.290

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Citation 1: Chen

Pursuant to 37 C.F.R. § 1.290(d)(2), the undersigned attorney provides a concise description of Chen *et al.*, "IL-11 receptor alpha in the pathogenesis of IL-13-induced inflammation and remodeling", 2005, J. Immunol. 174(4):2305-2313 ("Chen"). Pursuant to 37 C.F.R. § 1.290(d)(3), a copy of this reference is submitted. The above-identified application, U.S. Patent Application No. 15/381,622, was published on June 22, 2017 and a Requirement for Restriction/Election was mailed March 19, 2018; this Third Party Submission is being made pursuant to 37 C.F.R. § 1.290.

STATEMENT OF RELEVANCE OF CITATION

Chen teaches the pro-fibrotic activity of IL-11 including the relationship between IL-13 (known to be inflammatory and pro-fibrotic) and IL-11R α. The abstract states that "the relationships(s) between IL-11 and IL-13 in these responses has not been defined, and the role(s) of IL-11 in the genesis of the tissue effects of IL-13 has not been evaluated" (*see, e.g.*, Chen at p. 2305). The abstract further states that the authors "hypothesized that IL-11, signaling via the IL-11Rα-gp130 receptor complex, plays a key role in IL-13-induced tissue responses" (*see, e.g.*, Chen at p. 2305).

Chen provides data demonstrating that "IL-11 signaling plays a critical role in IL-13 induced tissue fibrosis and HA and myofibroblast accumulation" (*see, e.g.*, Chen at p. 2307, right column, middle paragraph). Chen stated that their studies were in agreement with prior studies of IL-11 and pulmonary fibrosis: "These findings are in accord with previous studies from our laboratory that demonstrated that transgenic IL-11 causes pulmonary fibrosis and myofibroblast accumulation" (*see, e.g.*, Chen at p. 2311, left column, bottom paragraph).

The last paragraph of Chen provides the following teaching and conclusion: "In our studies the ability of IL-13 to induce inflammation, fibrosis, HA accumulation, alveolar remodeling, and respiratory failure and death was decreased in mice that were deficient in IL-11Rα.…The decrease in IL-13 effector pathway activation could be caused by at least two mechanisms. First, IL-11 could be an important target of and mediator of the tissue effects of IL-13. Alternatively, IL-11 could regulate the survival of critical IL-13 target cells that are involved in the pathogenesis of IL-13-induced inflammation, remodeling, and cytokine, protease, and matrix responses. Regardless, exaggerated IL-13 production has been implicated in the pathogenesis of a variety of disorders, including asthma, COPD, pulmonary fibrosis, scleroderma, hepatic fibrosis, and nodular sclerosing Hodgkin's disease.…The present studies suggest that the effector response of IL-13 in these disorders may be beneficially controlled by interventions that block IL-11Rα and/or IL-11. This establishes the IL-11- IL-11Rα pathway as a worthwhile site for investigations designed to identify therapeutic agents that can be used to treat these and other IL-13-mediate disorders" (emphasis added) (*see, e.g.*, Chen at p. 2312, left column, bottom paragraph and right column).

Thus, Chen demonstrates an IL-11-dependent mechanism for the pro-fibrotic effects of IL-13 and suggests that interventions that block IL-11Rα and IL-11 would be beneficial for treating disorders including fibrotic disorders.

Chen is relevant to currently pending claims directed to methods of treating fibrosis, including independent claim 1 and dependent claims 21-31.

The undersigned believes that no fees are due with the instant Submission. However, the Commissioner is hereby authorized by this paper to charge any fees required by this Submission, including any fees required set forth in 37 C.F.R. § 1.290(f) to **Jones Day Deposit Account 50-3013**.

Respectfully submitted,

Date: April 30, 2018

/Janet M. McNicholas/
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Singapore Health Services PTE LTD et al.	Confirmation No.:	2829
Application No.:	15/381,622	Art Unit:	1646
Filing Date:	December 16, 2016	Examiner:	Prema Maria Mertz
Title:	TREATMENT OF FIBROSIS		

THIRD PARTY PREISSUANCE SUBMISSION UNDER 37 C.F.R. § 1.290

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Citation 2: Tang

Pursuant to 37 C.F.R. § 1.290(d)(2), the undersigned attorney provides a concise description of Tang *et al.*, "Targeted expression of IL-11 in the murine airway causes lymphocytic inflammation, bronchial remodeling, and airways obstruction", 1996, J. Clin. Invest. 98(12):2845-2853 ("Tang"). Pursuant to 37 C.F.R. § 1.290(d)(3), a copy of this reference is submitted. The above-identified application, U.S. Patent Application No. 15/381,622, was published on June 22, 2017 and a Requirement for Restriction/Election was mailed March 19, 2018; this Third Party Submission is being made pursuant to 37 C.F.R. § 1.290.

STATEMENT OF RELEVANCE OF CITATION

Tang teaches the pro-fibrotic activity of IL-11. The abstract states that: "Interleukin-11 is a pleotropic cytokine produced by lung stromal cells in response to respiratory viruses, cytokines, and histamine. To further define its potential effector functions, the Clara cell 10-kD protein promoter was used to express IL-11 and the airways of the resulting transgene mice were characterized" (*see, e.g.*, Tang at p. 2845).

The abstract provides the teaching and conclusion of the publication that: "These studies demonstrate that the targeted expression of IL-11 in the mouse airway causes a B and T cell–predominant inflammatory response, airway remodeling with increased types III and I collagen, the local accumulation of fibroblasts, myofibroblasts, and myocytes, and obstructive physiologic dysregulation" (*see, e.g.*, Tang at p. 2845).

Tang teaches that: "Chronic airway inflammation and airway remodeling (defined as fibrosis, matrix alterations, and/or changes in structural or resident cells of the airway wall) are important features of these disorders" citing multiple references (*see, e.g.*, Tang at p. 2845, right column, top paragraph).

Tang teaches that IL-11 causes fibrosis: "These studies demonstrate that IL-11 causes impressive airway alterations with transgene (+) animals manifesting a nodular B and T cell–predominant peribronchiolar inflammatory response, bronchial remodeling with subepithelial fibrosis, and physiologic dysfunction characterized by airways obstruction and nonspecific AHR" (*see, e.g.*, Tang at p. 2846, left column, first full paragraph; *see also*, *e.g.,* Figure 5, Figure 8 at pp. 2848-2849).

Tang teaches that IL-11 is fibrogenic: (a) "These studies….also demonstrate, for the first time, that IL-11 is a fibrogenic cytokine since it caused impressive airway remodeling with subepithelial fibrosis and the local accumulation of fibroblasts, myofibroblasts, and smooth muscle cells" (*see, e.g.*, Tang at p. 2851, left column, top paragraph); and (b) "This study demonstrates that IL-11 is a fibrogenic molecule and describes, for the first time, an animal model that reproduces many of the important features of the remodeled asthmatic airway. Specifically, it demonstrates that the targeted expression of IL-11 in the murine airway causes prominent subepithelial fibrosis with impressive increases in type III and to a lesser extent type I collagen. In addition, it demonstrates that this response occurs in the absence of overt basement membrane pathology and contains increased numbers of a variety of stromal cells, including airway myofibroblasts" (*see, e.g.*, Tang at p. 2851, right column, bottom paragraph).

The last paragraph of Tang provides the following teachings and conclusion: "In conclusion, our studies demonstrate that the transgenic expression of IL-11 in the mouse airway results in a nodular lymphocytic infiltrate, airway remodeling, and physiologic dysregulation that mimics in important ways the pathologic and physiologic features of viral and other airways disorders. These findings suggest that IL-11 may play an important role in the pathogenesis of the airways abnormalities seen in these often times devastating disorders. In this respect, CC10-IL-11 transgenic mice represent an excellent animal model of airway remodeling and inflammation that can be used to explore therapies directed at these processes and the contribution that these processes make to the physiologic abnormalities seen in a variety of human airways disorders" (*see, e.g.*, Tang at p. 2852, right column, bottom paragraph).

Tang is relevant to currently pending claims directed to methods of treating fibrosis, including independent claim 1 and dependent claims 21-31.

The undersigned believes that no fees are due with the instant Submission. However, the Commissioner is hereby authorized by this paper to charge any fees required by this Submission, including any fees required set forth in 37 C.F.R. § 1.290(f) to **Jones Day Deposit Account 50-3013**.

Respectfully submitted,

Date: April 30, 2018

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U.S. Patent and Trademark Office
Department of Commerce

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290
CONCISE DESCRIPTION OF RELEVANCE

Application Number	15381622

U.S. PATENTS

Cite No	Patent Number	Concise Description of Relevance

U.S. PATENT APPLICATION PUBLICATION

Cite No	Publication Number	Concise Description of Relevance

FOREIGN PATENT DOCUMENTS		
CiteNo	**Foreign Document Number**	**Concise Description of Relevance**

NON-PATENT PUBLICATIONS		
Cite No	**Reference**	**Concise Description of Relevance**
1	Chen et al., "IL-11 receptor alpha in the pathogenesis of IL-13-induced inflammation and remodeling", J Immunol., 174(4):2305-2313 (2005)	See Attached

2	Tang et al., "Targeted expression of IL-11 in the murine airway causes lymphocytic inflammation, bronchial remodeling, and airways obstruction", J Clin Invest., 98 (12):2845-2853 (1996)	See Attached

PTO/SB/429(08-12)
Approved for use through 07/31/2015. OMB 0651-0031
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THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	15381622

U.S. PATENTS				
Cite No	Patent Number	Kind Code[1]	Issue Date (YYYY-MM-DD)	First Named Inventor

U.S. PATENT APPLICATION PUBLICATIONS				
Cite No	Publication Number	Kind Code[1]	Publication Date (YYYY-MM-DD)	First Named Inventor

FOREIGN PATENTS AND PUBLISHED FOREIGN PATENT APPLICATIONS						
Cite No	Foreign Document Number[3]	Country Code[2]	Kind Code[1]	Publication Date (YYYY-MM-DD)	Applicant, Patentee or First Named Inventor	T[5]
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NON-PATENT PUBLICATIONS (e.g., journal article, Office action)			
Cite No	Author (if any), title of the publication, page(s) being submitted, publication date, publisher (where available), place of publication (where available).	T[5]	E[6]

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	15381622

1	Tang et al., "Targeted expression of IL-11 in the murine airway causes lymphocytic inflammation, bronchial remodeling, and airways obstruction", J Clin Invest., 98(12):2845-2853 (1996)	☐	☒
2	Chen et al., "IL-11 receptor alpha in the pathogenesis of IL-13-induced inflammation and remodeling", J Immunol., 174(4):2305-2313 (2005)	☐	☒

STATEMENTS

The party making the submission is not an individual who has a duty to disclose information with respect to the above-identified application under 37 CFR 1.56.

This submission complies with the requirements of 35 U.S.C. 122(e) and 37 CFR 1.290.

☐ The fee set forth in 37 CFR 1.290(f) has been submitted herewith.

☒ The fee set forth in 37 CFR 1.290(f) is not required because this submission lists three or fewer total items and, to the knowledge of the person signing the statement after making reasonable inquiry, this submission is the first and the only submission under 35 U.S.C 122(e) filed in the above-identified application by the party making the submission or by a party in privity with the party.

☐ This resubmission is being made responsive to a notification of non-compliance issued for an earlier filed third-party submission. The corrections in this resubmission are limited to addressing the non-compliance. As such, the party making this resubmission: (1) requests that the Office apply the previously-paid fee set forth in 37 CFR 1.290(f), or (2) states that no fee is required to accompany this resubmission as the undersigned is again making the fee exemption statement set forth in 37 CFR 1.290(g).

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	15381622

Signature	/Janet M. McNicholas/		
Name/Print	Janet M. McNicholas	Registration Number (if applicable)	32918

Examiner Signature		Date Considered	

*EXAMINER: Signature indicates all documents listed above have been considered,except for citations through which a line is drawn. Draw line through citation if not considered. Include a copy of this form with next communication to applicant. 1. If known, enter kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16. See MPEP 901.04(a). 2. Enter the country or patent office that issued the document, by two-letter code under WIPO standard ST.3. See MPEP 1851. 3. For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. 4. If known, enter the kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 . See MPEP 901.04(a). 5. Check mark indicates translation attached. 6. Check mark indicates evidence of publication attached.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Gregory, Adam
MEWBURN ELLIS LLP
City Tower
40 Basinghall Street
London
Greater London EC2V 5DE
ROYAUME UNI

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	06.11.2017
Applicant's or agent's file reference RIC/FP7230618	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2016/081430	16.12.2016	16.12.2015

Applicant
Singapore Health Services Pte Ltd

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary report on patentability and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary report on patentability. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Fax +49 89 2399 - 4465	Marra, Emanuela Tel +49 89 2399-7235

Form PCT/IPEA/416 (January 2004)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference RIC/FP7230618	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No PCT/EP2016/081430	International filing date *(day/month/year)* 16.12.2016	Priority date *(day/month/year)* 16.12.2015

International Patent Classification (IPC) or national classification and IPC
INV. C07K16/24

Applicant
Singapore Health Services Pte Ltd

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 10 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and/or sheets containing rectifications authorized by this Authority, unless those sheets were superseded or cancelled, and any accompanying letters (see Rules 46.5, 66.8, 70.16, 91.2, and Section 607 of the Administrative Instructions).
      ☐ sheets containing rectifications, where the decision was made by this Authority not to take them into account because they were not authorized by or notified to this Authority at the time when this Authority began to draw up this report, and any accompanying letters (Rules 66.4bis, 70.2(e), 70.16 and 91.2).
      ☐ superseded sheets and any accompanying letters, where this Authority either considers that the superseding sheets contain an amendment that goes beyond the disclosure in the international application as filed, or the superseding sheets were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in item 4 of Box No. I and the Supplemental Box (see Rule 70.16(b)).
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing, in the form of an Annex C/ST.25 text file, as indicated in the Supplemental Box Relating to Sequence Listing (see paragraph 3*ter* of Annex C of the Administrative Instructions).

4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☒ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
14.08.2017	06.11.2017

Name and mailing address of the international preliminary examining authority.	Authorized officer
European Patent Office D-80298 Munich Tel +49 89 2399 - 0 Fax. +49 89 2399 - 4465	Page, Michael Telephone No +49 89 2399-7322

Form PCT/IPEA/409 (cover sheet) (January 2015)

Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:

   - ☐ international search (under Rules 12.3(a) and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a) and (b))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-93	as originally filed	

Sequence listings, SEQ ID NO

1-77	as originally filed	

Claims, Numbers

1-14	filed in electronic form on	14-08-2017

Drawings, Sheets

1/66-66/66	as originally filed	

   ☒ a sequence listing - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since either they are considered to go beyond the disclosure as filed, or they were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in the Supplemental Box (Rules 70.2(c) and (c-*bis*)):

   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:

5. ☐ This report has been established:
   - ☐ taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rules 66.1(d-*bis*) and 70.2(e)).
   - ☐ without taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91(Rules 66.4*bis* and 70.2(e)).

6. ☒ With regard to top-up searches (Rules 66.1*ter* and 70.2(f)):
   - ☒ A top-up search was carried out by this Authority on 09.10.2017 (all discovered documents are listed in the Supplemental Box Relating to Top-up Search).
     - ☐ Additional relevant documents have been discovered during the top-up search.
   - ☐ No top-up search was carried out by this Authority because it would serve no useful purpose.

7. ☐ Supplementary international search report(s) from Authority(ies) has/have been received and taken into account in establishing this report (Rule 45bis.8(b) and (c)).

* *If item 4 applies, some or all of those sheets may be marked "superseded".*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-14
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-14
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-14
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of a sequence listing:

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

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

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

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

   c. ☐ furnished subsequent to the international filing date for the purposes of international search and/or examination:

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

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

   d. ☐ furnished to this Authority as an amendment* under PCT Article 34 on :

      ☐ in the form of an Annex C/ST.25 text file, and preferably identified as "Amended" at the first line of text.

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

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

3. Additional comments:

* *If item 4 in Box No. I applies, the sequence listing, which forms part of the basis of the report, may be marked "superseded."*

1 Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1.1 **Reference is made to the following documents:**

D1 M. OBANA ET AL: "Therapeutic Activation of Signal Transducer and Activator of Transcription 3 by Interleukin-11 Ameliorates Cardiac Fibrosis After Myocardial Infarction",
CIRCULATION,
vol. 121, no. 5, 9 February 2010 (2010-02-09), pages 684-691, XP055359357,
ISSN: 0009-7322, DOI: 10.1161/CIRCULATIONAHA.109.893677

D2 M Stangou ET AL: "Effect of IL-11 on glomerular expression of TGF-beta and extracellular matrix in nephrotoxic nephritis in Wistar Kyoto rats",
J Nephrol, 1 January 2011 (2011-01-01), pages 106-111, XP055359363,
Retrieved from the Internet:
URL:http://www.ncbi.nlm.nih.gov/pubmed/20640990
[retrieved on 2017-03-28]

D3 US 2010/093976 A1 (AZUMA JUNICHI [JP] ET AL) 15 April 2010 (2010-04-15)

D4 AHROM HAM ET AL: "Critical Role of Interleukin-11 in Isoflurane-mediated Protection against Ischemic Acute Kidney Injury in Mice",
ANESTHESIOLOGY.,
vol. 119, no. 6, 1 December 2013 (2013-12-01), pages 1389-1401, XP055359340,
PHILADELPHIA, PA, US
ISSN: 0003-3022, DOI: 10.1097/ALN.0b013e3182a950da

D5 WO 98/36061 A2 (UNIV MANCHESTER [GB]; FERGUSON MARK WILLIAM JAMES [GB]; KANE SHARON O) 20 August 1998 (1998-08-20)

D6 WO 00/78336 A1 (GENETICS INST [US]; UNIV JOHNS HOPKINS [US]) 28 December 2000 (2000-12-28)

1.2 **Novelty - Art.33(1) and (2) PCT:**

Antagonists of IL-6 and IL-13 for preventing fibrosis are known in the art (D5, D6). Not, however, IL-11.

1.3 **Inventive Step - Art.33(1) and (3) PCT:**

D5 and D6 are both considered to be equivalent closest prior art in that they solve the same technical problem; prevention of fibrosis through antagonism of pro-fibrotic interleukins (claims 1-23), determination of patients suitable for treatment with the same (claims 24 and 25) diagnosis (claims 26-28, 31 and 32) or prognosis (claims 29 and 30).

D1-D4 all teach away from the present solution, as they all indicate that human IL-11 prevents fibrosis in heart tissue and kidney in animal experiments.

By contrast, Examples 2, 3 and 5 document that IL-11 is pro-fibrotic in a number of *in vitro* assays involving cultured cells.

Supplementary experimental evidence provided by the Applicant, including arguments from D1, provides conflicting evidence regarding whether IL-11 is a pro- or anti-fibrotic factor. The veracity of the supplemental data will be subjected to more intense scrutiny in the regional phase. For now, however, the Applicant is given the benefit of the doubt that, at least under certain circumstances, IL-11 is a pro-fibrotic factor and that therefore its antagonism might lead to a reduction in fibrosis.

Given the apparent prejudice created by D1-D4, the solution proposed by the Applicant is provisionally regarded as demonstrating inventive step.

2 **Re Item VIII**

Certain observations on the international application

2.1 **Claims 4-14** are all mixed category claims and as such are not considered to meet the clarity requirements of Article 6 PCT. In order to be clear, claims should pertain to a method or a product, but not both.

2.2 No unified criteria exist in the PCT Contracting States on the question whether methods of treatment are industrially applicable, as they are not considered to be industrially applicable in the EPC. No opinion can be given, therefore, on the industrial applicability of **claims 3-14**, which all claim such methods.

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Eliot Ward
Rhiannon Wescott

European Patent Office
D-80298 München
Germany

14 August 2017

FILED ELECTRONICALLY

BEFORE THE EUROPEAN PATENT OFFICE (EPO)
ACTING AS THE
INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY (IPEA)

Dear Sirs

International Patent Application No. PCT/EP2016/081430
Applicant: SINGAPORE HEALTH SERVICES PTE LTD et al.
Our Ref: APG/FP7230618

This letter accompanies the Chapter II Demand filed in connection with this application.

1. Fees

The following fees are being paid online today:

PCT Preliminary Examination Fee	Euro	1930
PCT Handling Fee	Euro	183
TOTAL:	Euro	2113

If any additional fees are required so that this Chapter II Demand is deemed to be filed, then the EPO is authorised to deduct such fees from my firm's deposit account number 2805.0013 under the reference SHORTFALL, informing me in writing that this has been done.

2. Amendments

Amended claims 1 to 14 are filed under Article 34 PCT on replacement pages 94 and 95. For the examiner's convenience a marked up version is included for reference only.

Independent claims 1, 2 and 3 are amended to specify that the agent is an antibody capable of binding to Interleukin 11 (IL-11) or IL-11 receptor α (IL-11Rα), and is capable of inhibiting IL-11 mediated signalling. Basis for the amendment comes from, e.g. page 4, lines 4-13; page 9, line 33; and page 10, lines 19 to 22 of PCT/EP2016/081430 as filed.

Claims 1, 2 and 3 are also amended to specify use treating/preventing fibrosis in a human. Basis comes from e.g. page 42, lines 2-5.

Claim 5 is amended for conformity with claims 1, 2 and 3 as currently amended.

Previous claims 6 to 8 are deleted.

Claim 6 (previous claim 9) is amended for conformity with claims 1, 2 and 3 as currently amended, and also to specify IL-11Rα. Basis comes from, e.g. page 9, line 33.

Previous claims 10 to 15 are deleted.

Claims 7 to 14 (previous claims 16 to 23) are amended editorially for improved readability, and for conformity with amendments to the claims from which they depend.

Previous claims 24 to 34 are deleted.

It is hereby submitted that the claim amendments do not extend beyond the disclosure of the international application as filed, in accordance with Article 34(2)(b) PCT, second sentence.

3. Novelty

I thank the Examiner for recognising that the use of antagonists of IL-11 for preventing fibrosis is not known in the art, and for acknowledging the novelty for the claims.

4. Inventive Step

The Examiner comments that the subject-matter of the claims is not considered to be inventive as the technical problem of treating/preventing fibrosis is not solved.

The Examiner considers that the prior art shows that IL-11 prevents fibrosis in heart and kidney tissue, and so an agent capable of inhibiting the action of IL-11 would not be useful to treat/prevent fibrosis.

Favourable reconsideration of the objection is respectfully requrested in view of the following submissions.

4.1 Prior art studies relating to the role of IL-11

Obana et al., Circulation (2010) 121(5):684-691 (**D1**) is cited by the Examiner as evidence that IL-11 prevents fibrosis in heart tissue. **D1** discloses that treatment with IL-11 reduced the fibrosis area 14 days after myocardial infarction (MI) in a mouse model of MI by coronary ligation (see e.g. Abstract).

Importantly, in the experiments reported in **D1** mice were treated with recombinant human IL-11 – see page 685, left column, paragraph entitled 'Coronary Artery Ligation and IL-11 Treatment', final sentence:

> *"In the IL-11 group, recombinant **human** IL-11 (Peprotech) was administered intravenously for 5 days consecutively; the control group received the same volume of phosphate-buffered saline (PBS) during the same period."* [emphasis added]

Stangou et al., J Nephrol (2011) 106-111 (**D2**) is also cited by the Examiner. This document discloses suppression of glomerular expression of TGFβ1 and extracellular matrix deposition in rat models of glomerulonephritis following administration of a high dose of recombinant human IL-11 (i.e. rhIL-11; see e.g. page 106, right column, second sentence).

The Abstract to **D2**, 'Methods; and 'Results' provide:

> *"Methods: Following induction of nephrotoxic nephritis, expression of TGF-β1, α-smooth muscle actin (α-SMA), fibronectin and p-p38 MAPK was detected in the kidney.* ***Rats were treated*** *either with vehicle or* ***rhIL-11*** *at a high or low dose and culled on day 6.*
> *Results: A high dose of* ***rhIL-11*** *resulted in a significant reduction in the glomerular expression of TGF-β1 (0.4 ± 0.1 vs. 2.04 ± 0.4 semiquantitative score, p<0.005), α-SMA (0.6 ± 0.2 vs. 1.5 ± 0.3, p<0.01) and fibronectin (0.6 ± 0.1 vs. 1.5 ± 0.1, p<0.02). The periglomerular expression of α-SMA and fibronectin was significantly reduced in rats treated with the high dose of* ***rhIL-11*** *(9.6% ± 2% vs. 92% ± 2.5% of glomeruli, p<0.01; and 26% ± 4.9% vs. 94% ± 1.9% of glomeruli, p<0.005, respectively). There was a slight but insignificant reduction of p-p38 MAPK in IL-11 treated rats. Treatment with low-dose rhIL-11 did not reduce expression of these molecules."*
> [emphasis added]

US 2010/093976 A1 (**D3**) cited by the Examiner is the publication of a US patent application from the research group of **D1** (led by Prof. Yasushi Fujio at Osaka University). Paragraphs [0085]-[0087] disclose that in a myocardial infarction model, mice treated with IL-11 had a reduced infarct area (measured as the proportion of fibrotic area in the left ventricular myocardium), as compared to PBS-treated mice.

Whilst the species of IL-11 used in **D3** is not disclosed, it appears highly likely that this is human IL-11. Paragraph [0058] discloses that the IL-11 was obtained from PeproTech, which is the same source as the recombinant human IL-11 used by the same researchers for the experiments of **D1**. Furthermore, Kimura et al., Cytokine (2007) 38(2):107-15 (Kimura; enclsoed), is another publication by the same research group which describes many of the experiments and results disclosed in **D3** – compare Figs. 2-4 of **D3** with Figs. 3a-c of Kimura; Fig. 5 of **D3** with Fig. 6 of Kimura; and Fig. 9 of **D3** with Fig. 1 of Kimura.

Page 108, left column, last complete paragraph of Kimura makes clear that the experiments used recombinant human IL-11, which was again purchased from Peprotech.

Therefore, each of **D1** to **D3** report the results of studies which are fundamentally flawed in their design, for seeking to analyse the role of IL-11 in pathophysiological processes by analysing effects following administration of the human IL-11 molecule in rodent models of disease processes.

The effects observed in these studies following administration of human IL-11 cannot be separated from, for example, the mouse/rat host response to this non-host molecule.

Furthermore, it cannot be assumed that effects observed for human IL-11 in mouse and rat would accurately reflect the role of human IL-11 in fibrosis in humans. Human IL-11, IL11-Rα and gp130 molecules have distinct amino acid sequences to their mouse and rat homologues, and so it cannot be assumed that human IL-11 would interact with mouse and rat receptor molecules to effect the same outcomes as would be observed in humans.

This is further supported by Figure 20F of the present application, which shows that mouse fibroblasts are considerably more sensitive to activation *in vitro* to a pro-fibrotic myofibroblast phenotype by treatment with murine IL-11 as compared to treatment with human IL-11 (see accompanying legend on page 55).

Ham et al., Anesthesiology (2013) 119(6): 1389-1401 (**D4**) is also cited by the Examiner. This document reports induction of IL-11 mRNA and protein expression by human kidney proximal tubule cells and mouse kidney cells cultured *in vitro*, and by mouse kidney *in vivo*, following treatment with isofluorane.

Figures 6-9 are disclosed to show protective effects for IL-11 following ischemic acute kidney injury, using IL-11R WT and knockout mice. We note that these assays evaluate correlates of renal function, necrosis, neutrophil infiltration and apoptosis. None of the experiments of **D4** investigate the effect of IL-11 in fibrosis.

It will be clear from the foregoing explanation that it is not possible to draw any scientifically meaningful conclusions as to the role of IL-11 in fibrosis from **D1** to **D4.**

Moreover, **Annex I** filed herewith provides further evidence that the experiments performed in **D1** are invalid, and that conclusions drawn from the results are fallacious.

Annex I reports the results of studies in which myocardial infarct was generated in mice by coronary artery ligation, or sham-operated, and wherein the mice were then treated either with PBS or with recombinant mouse IL-11.

Histological analysis of heart tissue sections revealed an increase in several markers of the fibrosis associated with IL-11 treatment (Figure 1). Figure 1A of **Annex I** shows increased collagen deposition (a known indicator of fibrosis) as determined by Masson's trichrome staining. Figure 1A also reveals upregulated expression of αSMA (as measured by ACTA2 staining) – a marker of fibroblast activation. Activated fibroblasts are known to be the central effectors of the fibrotic response post-myocardial infarction, and so the level of ACTA2 is an extremely reliable indicator of the fibrotic response. Figures 1B and 1C reveal that IL-11 treatment was associated with a statistically significant increase in epicardial thickness at the border of the infarct and at the right ventricle, whilst Figures 1D and 1E show IL-11 treatment caused a statistically significant reduction in heart function, as determined by analysis of the ejection fraction and fractional shortening.

Annex I indicates that treatment of mice with mouse IL-11 following myocardial infarction increases fibrosis, and is associated with a reduction in heart function.

4.2 The data of the present application

The present application provides a wealth of data demonstrating a pro-fibrotic role for IL-11, and establishing the therapeutic utility of antagonists of IL-11 mediated signalling in the treatment/prevention of fibrosis.

Some of the key data are highlighted below.

Example 2 at page 60, third paragraph and Figures 7A and 7B demonstrate that incubation of primary human atrial fibroblasts with recombinant human IL-11 increases deposition of collagen by fibroblasts, a well-established fibrotic process. Moreover, treatment with neutralising anti-IL-11 antibody (but not isotype control antibody) is shown to abrogate

collagen production induced by stimulation of the fibroblasts with TGFβ1 (a known pro-fibrotic stimulus).

Example 3 at page 61, and Figure 10 further demonstrate the ability of neutralising anti-IL-11 antibody to abrogate increased collagen production by human atrial fibroblasts in response to various other pro-fibrotic stimuli (ANG2, PDGF, ET-1).

Example 5.2 at pages 63-64 and Figures 20A-20E provide further data supporting a pro-fibrotic role for IL-11 in heart tissue. Human atrial fibroblasts were shown to display significantly increased production of extracellular matrix components (collagen, periostin) and increased expression of pro-fibrotic markers (αSMA, IL-6, MMP2, TIMP1) in response to treatment with human IL-11 protein, in the same way as production of these factors is increased in response to treatment with the pro-fibrotic stimulus TGFβ1.

Example 5.3.1 and Figures 38A to 38D likewise show increased production of extracellular matrix components and increased expression of fibrotic markers by human primary liver fibroblasts in response to treatment with human IL-11, and also the ability of neutralising anti-IL-11 antibody to abrogate the profibrotic effects of stimulation with TGFβ1.

Figures 22A to 22F and 23A and 23B show that TGFβ1-mediated fibrosis can be inhibited by treatment with neutralising anti-IL-11 antibody. Figure 24 moreover shows that IL-11-binding decoy receptor molecules, neutralising anti-IL-11Rα antibodies and oligonucleotides encoding siRNA for antisense knockdown of *IL-11* and *IL-11RA* gene expression are similarly able to inhibit TGFβ1-mediated transition of fibroblasts to myofibroblasts (fibrosis effector cells). Further data showing inhibition of the TGFβ1-mediated fibrotic response using decoy IL-11 receptors is provided at Figures 32A and 32B.

Example 5.3.3 at page 64-65 and Figure 21B and 21C provide *in vivo* data demonstrating IL-11 to be pro-fibrotic in a variety of tissues. Injection of mice with recombinant mouse IL-11 caused an increase in the relative weight of heart, kidney, lung and liver (Figure 21B), and that this was associated with increased collagen content in these tissues (Figure 21C).

Further *in vivo* data supporting a pro-fibrotic role for IL-11 is provided at Examples 7.2 and 7.3, and Figures 27A to 27D and Figure 28. These experiments show that IL-11RA knockout mice are protected from fibrosis of the heart and kidney tissues induced by profibrotic stimuli, indicating signalling through the IL-11 receptor as an important mediator of fibrotic processes. Further still, Figures 31A and 31B, summarised at the legend to Figure 31 on page 57 – more fibrosis was detected in eye sections obtained from wildtype mice than IL-11RA knockout mice at 7 days following trabeculectomy.

Thus the present application provides abundant data from both *in vitro* and *in vivo* studies proving that IL-11/IL-11R signalling is a key mediator of fibrosis in a wide range of tissues, and demonstrates that inhibition of IL-11 mediated signalling reduces fibrosis, as determined by analysis of a variety of markers of the fibrotic response.

4.3 Inventive step

At section 1.3 of the WO/ISA the Examiner identifies **D5** and **D6** as being equivalent closest prior art to the present invention, each relating to prevention of fibrosis through antagonism of pro-fibrotic interleukins.

Neither of **D5** nor **D6** disclose treatment of fibrosis using an antibody which is capable of binding to IL-11 or IL-11Rα and inhibiting IL-11 mediated signalling (cf. independent claims 1, 2 and 3).

Starting from either of **D5** or **D6**, the technical problem to be solved by the present invention can be formulated as "the provision of an alternative treatment for the prevention or treatment of fibrosis".

The claimed solution to use an agent which is capable of binding to IL-11 or IL-11Rα and inhibiting IL-11 mediated signalling would not have been obvious to the skilled person at the relevant date.

As explained in the present application e.g. at page 1, line 31 to page 2 line 24, the role of IL-11 in fibrosis was not clear at the priority date for the present application, and the majority of studies suggested that IL-11 is anti-fibrotic, as evidenced e.g. by **D1** to **D3** identified by the Examiner.

In view of the state of the art at the relevant date, the skilled person simply would not have arrived at the subject-matter of the present independent claims. In view of the uncertainty as the role of IL-11 in fibrosis, the skilled person would not have considered to use antagonists of IL-11 mediated signalling to treat/prevent fibrosis with a reasonable expectation of success.

Rather, only the extensive data of the present application from *in vitro* and *in vivo* studies described under section 4.2 above make plausible the use of antagonists of IL-11 mediated signalling for the treatment or prevention of fibrosis.

Accordingly, the subject-matter of independent claims 1 to 3, and therefore the claims dependent therefrom, involve an inventive step over the cited prior art documents (Article 33(3) PCT).

5. Support

At section 2.1 of the WO/ISA the Examiner objects that the claims lack support, as evidenced by **D1** to **D3**.

Favourable reconsideration of the objection is respectfully requested in view of sections 4.1 and 4.2 above, which explain failings of the prior art studies in relation to the role of IL-11 in fibrosis, and which make clear that the present application supports the therapeutic utility of anti-IL-11 and anti-IL-11Rα antibody antagonists of IL-11 mediated signalling to treat/prevent fibrosis.

6. Clarity

6.1 Structural nature of the agent

The Examiner objects at section 2.2 of the WO/ISA that the claims lack clarity in respect of the structural nature of the agent used to inhibit the action of IL-11.

The independent claims are currently amended to specify an antibody which is capable of binding to IL-11 or IL-11Rα and inhibiting IL-11 mediated signalling.

As explained in section 4.2 above, the application demonstrates the use of anti-IL-11 antibodies and anti-IL-11Rα antibodies to bind to IL-11/IL-11Rα and inhibit IL-11 mediated signalling (see e.g. Figure 24).

The skilled person is able to determine whether a given anti-IL-11 antibody or anti-IL-11Rα is capable of inhibiting IL-11 mediated signalling with certainty and without undue burden.

6.2 Claims depending from claims in different categories

At section 2.3 of the WO/ISA the Examiner objects that certain dependent claims lack clarity for depending from claims in different categories.

Favourable reconsideration of the objection is respectfully requested in view of the present amendments to the claims and the following comments.

The objection pertains to claims which depend from independent claims relating to methods of treatment and medical uses, drafted in accordance with the different practices of national/regional patent offices relating to the allowability of claims defining methods of treatment, and allowable medical use claim formats.

The dependent claims specify further features of the claim in the appropriate format, as is clear from the preamble to the claims. There is no lack of clarity.

Take the example of present claim 4:

> *"4. The antibody for use in a method of treating or preventing fibrosis according to claim 1, the use according to claim 2, or the method according to claim 3, wherein the antibody is capable of preventing or reducing the binding of IL-11 to an IL-11 receptor."*

This claim clearly specifies further features of the antibody specified in respective medical use and method of treatment claims, and the skilled person is left with no uncertainty in this respect.

All claims satisfy the requirements of Article 6 PCT.

7. Industrial applicability – methods of treatment

At section 2.4 the Examiner comments that no opinion can be given in respect of the industrial applicability of claims relating to methods of treatment.

The applicant intends to address this issue as necessary before the different offices during the national/regional phase.

8. Closing Remarks

It is requested that an International Preliminary Report on Patentability (IPRP/Chapter II) be issued indicating that all claims are novel and inventive.

In the event that the IPEA has any outstanding objections we request a telephone interview with the Examiner in accordance with Article 34(2)(a) and Rule 66.6 PCT.

Yours faithfully

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Encs. Article 34 Amendments
Copy showing changes
Kimura et al., Cytokine (2007) 38(2):107-15
Annex I

APG/krg

Claims:

1. An antibody which is capable of binding to Interleukin 11 (IL-11) or IL-11 receptor α (IL-11Rα) and inhibiting IL-11 mediated signalling, for use in a method of treating or preventing fibrosis in a human.

2. Use of an antibody which is capable of binding to Interleukin 11 (IL-11) or IL-11 receptor α (IL-11Rα) and inhibiting IL-11 mediated signalling, in the manufacture of a medicament for use in a method of treating or preventing fibrosis in a human.

3. A method of treating or preventing fibrosis in a human, the method comprising administering to a subject in need of treatment a therapeutically effective amount of an antibody which is capable of binding to Interleukin 11 (IL-11) or IL-11 receptor α (IL-11Rα) and inhibiting IL-11 mediated signalling.

4. The antibody for use in a method of treating or preventing fibrosis according to claim 1, the use according to claim 2, or the method according to claim 3, wherein the antibody is capable of preventing or reducing the binding of IL-11 to an IL-11 receptor.

5. The antibody for use in a method of treating or preventing fibrosis according to claim 1 or 4, the use according to claim 2 or 4, or the method according to claim 3 or 4, wherein the antibody is an IL-11 binding antibody.

6. The antibody for use in a method of treating or preventing fibrosis according to claim 1 or 4, the use according to claim 2 or 4, or the method according to claim 3 or 4, wherein the antibody is an IL-11Rα binding antibody.

7. The antibody for use in a method of treating or preventing fibrosis according to any one of claims 1 or 4 to 6, the use according to any one of claims 2 or 4 to 6, or the method according to any one of claims 3 or 4 to 6, wherein the fibrosis is fibrosis of the heart, liver, kidney or eye.

8. The antibody for use in a method of treating or preventing fibrosis according to any one of claims 1 or 4 to 7, the use according to any one of claims 2 or 4 to 7, or the method according to any one of claims 3 or 4 to 7, wherein the fibrosis is in the heart and

is associated with dysfunction of the musculature or electrical properties of the heart, or thickening of the walls or valves of the heart.

9. The antibody for use in a method of treating or preventing fibrosis according to any one of claims 1 or 4 to 7, the use according to any one of claims 2 or 4 to 7, or the method according to any one of claims 3 or 4 to 7, wherein the fibrosis is in the liver and is associated with chronic liver disease or liver cirrhosis.

10. The antibody for use in a method of treating or preventing fibrosis according to any one of claims 1 or 4 to 7, the use according to any one of claims 2 or 4 to 7, or the method according to any one of claims 3 or 4 to 7, wherein the fibrosis is in the kidney and is associated with chronic kidney disease.

11. The antibody for use in a method of treating or preventing fibrosis according to any one of claims 1 or 4 to 7, the use according to any one of claims 2 or 4 to 7, or the method according to any one of claims 3 or 4 to 7, wherein the fibrosis is in the eye and is retinal fibrosis, epiretinal fibrosis, or subretinal fibrosis.

12. The antibody for use in a method of treating or preventing fibrosis according to any one of claims 1 or 4 to 11, the use according to any one of claims 2 or 4 to 11, or the method according to any one of claims 3 or 4 to 11, wherein the method of treating or preventing comprises administering said antibody to a subject in which IL-11 or IL-11R expression is upregulated.

13. The antibody for use in a method of treating or preventing fibrosis according to any one of claims 1 or 4 to 12, the use according to any one of claims 2 or 4 to 12, or the method according to any one of claims 3 or 4 to 12, wherein the method of treating or preventing comprises administering said antibody to a subject in which IL-11 or IL-11R expression has been determined to be upregulated.

14. The antibody for use in a method of treating or preventing fibrosis according to any one of claims 1 or 4 to 13, the use according to any one of claims 2 or 4 to 13, or the method according to any one of claims 3 or 4 to 13, wherein the method of treating or preventing comprises determining whether IL-11 or IL-11R expression is upregulated in the subject and administering said antibody to a subject in which IL-11 or IL-11R expression is upregulated.

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A Fusion Protein of the gp130 and Interleukin-6Rα Ligand-binding Domains Acts as a Potent Interleukin-6 Inhibitor*

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Interleukin (IL)-6 is involved in the maintenance and progression of several diseases such as multiple myeloma, rheumatoid arthritis, or osteoporosis. The present work aims at the development of an IL-6 inhibitor for the use in anti-cytokine therapies. The IL-6 receptor is composed of two different subunits, an α-subunit (IL-6Rα) that binds IL-6 with low affinity and a β-subunit (gp130) that binds the IL-6·IL-6Rα complex with high affinity and as a result triggers intracellular signaling. In its soluble form, gp130 is a natural antagonist that neutralizes IL-6·soluble IL-6Rα complexes. It was our strategy to appropriately fuse the two receptor subunit fragments involved in IL-6 receptor complex formation to bind IL-6 with high affinity and to antagonize its effects. The ligand-binding domains of gp130 (D1-D2-D3) and IL-6Rα (D2-D3) were connected using three different linkers. The resulting constructs were expressed in stably transfected insect cells and tested for their ability to inhibit IL-6 activity in several *in vitro* systems. All fusion proteins were strong inhibitors of IL-6 signaling and abrogated IL-6-induced phosphorylation of STAT3, proliferation of transfected Ba/F3 cells, and induction of acute-phase protein synthesis. As intended, the fused receptors were much more effective than the separately expressed soluble receptor proteins. The fusion protein strategy presented here can also be applied to other cytokines that signal via receptors composed of two different subunits to design new potent inhibitors for anti-cytokine therapies.

Anti-cytokine therapies are aimed at the inhibition of a certain cytokine that is responsible for the maintenance of a disease. Different strategies have been used to neutralize cytokines in patients. Most effective has been the application of soluble cytokine receptors that consist solely of the ectodomain but lack the transmembrane and cytoplasmic regions. They bind the respective cytokine with high affinity and specificity as membrane-bound receptors do. In the treatment of chronic inflammatory diseases such as rheumatoid arthritis, the use of dimeric soluble tumor necrosis factor receptors for the neutralization of tumor necrosis factor has been a real breakthrough (1).

IL-6[1] is secreted by several cell types in response to various inflammatory stimuli. It is the major mediator of the acute-phase response of the liver and is involved in the coordination of inflammatory and immune responses at the site of inflammation (2). In several acute and chronic inflammatory diseases such as rheumatoid arthritis and inflammatory bowel diseases, in postmenopausal osteoporosis, but also in certain types of cancer, IL-6 levels are elevated and a causal role for IL-6 in disease progression has been suggested. In some cases inhibition of IL-6 activity by receptor antagonists or neutralizing antibodies has beneficial effects (3, 4).

IL-6 belongs to the family of hematopoietic cytokines (5). It is a member of the subfamily of IL-6-type cytokines (6) comprising IL-6, IL-11, ciliary neurotrophic factor, leukemia inhibitory factor, oncostatin M, cardiotrophin-1, and cardiotrophin-like cytokine. They all use the hematopoietic cytokine receptor gp130 as a common signal-transducing receptor subunit (7). As a result of receptor activation the transcription factor STAT3 becomes tyrosine-phosphorylated and translocates into the nucleus to induce target gene expression (8, 9).

Expression of gp130 is not sufficient for cells to become responsive to IL-6. They additionally have to express the cytokine specific α-receptor subunit IL-6Rα. This α-receptor is not involved in the initiation of the cytoplasmic signal transduction cascades but is essential for cytokine binding. Thus, activation of the receptor by IL-6 requires two steps: (i) low affinity IL-6 binding to IL-6Rα and (ii) subsequent recruitment of the complex of IL-6 and IL-6Rα to two gp130 molecules leading to the formation of a high affinity ternary complex (10).

Cells lacking IL-6Rα can be stimulated with the combination of IL-6 and soluble IL-6Rα (sIL-6Rα) (10). In such a situation, IL-6 binds to sIL-6Rα in solution and the heterodimer of IL-6/sIL-6Rα activates membrane-bound gp130. Soluble gp130 (sgp130) alone acts as a relatively weak IL-6 antagonist (11). Most interestingly, the antagonizing activity of sgp130 is substantially increased by the presence of sIL-6Rα (12). Both sIL-6R (13, 14) and sgp130 (11, 12) are found in high concentrations in human blood (about 50 and 300 ng/ml, respectively). This pair of soluble receptors might act as a natural IL-6 inhibitor to limit systemic IL-6 responses (12).

Structurally, IL-6 belongs to the family of the α-helix-bundle cytokines. IL-6Rα as well as gp130 belong to the family of class I cytokine receptors (5). The extracellular regions of IL-6Rα and gp130 consist of three (D1–D3) (15) or six domains (D1–D6) (16), respectively. D2 and D3 of IL-6Rα are involved in IL-6

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[1] The abbreviations used are: IL, interleukin; D, domain; fp, fusion protein; s, soluble; STAT, signal transducer and activator of transcription; ELISA, enzyme-linked immunosorbent assay.

binding (17). The complex of IL-6 and IL-6Rα is bound by D1–D3 of gp130 (18). IL-6 contains three receptor-binding sites. Site I is occupied by D2-D3 of IL-6Rα, and sites II and III bind to D2-D3 and D1 of gp130, respectively (19–21). Based on the mutagenesis data and the recently solved structure of D1–D3 of gp130 bound to viral IL-6, which binds gp130 in the absence of any α-receptor, a reliable model of the IL-6·IL-6Rα·gp130 ternary complex has been proposed (22).

Inhibition of IL-6 activity by the use of soluble receptors is challenging because of the bipartite nature of the IL-6 receptor. IL-6 alone does not bind to gp130. To be neutralized by sgp130, IL-6 must first bind to sIL-6Rα. A fusion protein of gp130 and sIL-6Rα would therefore guarantee that the agonistic complex of IL-6·sIL-6Rα is immediately neutralized. Only recently, due to the new structural data on the IL-6·receptor complex (22), a promising rational approach on how to design an IL-6-antagonist based on a fusion of sgp130 with sIL-6Rα became possible. In this study, we present a highly potent IL-6 antagonist consisting of the ligand-binding moieties of sgp130 and sIL-6Rα.

EXPERIMENTAL PROCEDURES

Cloning of the Fusion Proteins—A fragment corresponding to D2-D3 of IL-6Rα (Val^{110}–Lys^{338}) was amplified by PCR introducing a multiple cloning site (*Sma*I, *Not*I, MluI, *Nhe*I) with the sense primer and a *Apa*I site, a stop codon instead of Met^{331}, and a *Bam*HI site with the antisense primer. The product was cut with *Sma*I (Roche Diagnostic GmbH, Mannheim, Germany) and *Bam*HI (MBI Fermentas GmbH, St. Leon-Rot, Germany) and cloned into pSVL-gp130 (D1–D3) (Met^{1}–Pro^{326}) digested with the same enzymes. Then three different linkers were added after digestion of the obtained chimeric construct with *Mlu*I (Promega, Madison, WI) and *Nhe*I (MBI Fermentas GmbH). The first linker (stalk-49) corresponding to the short extracellular membrane proximal part of IL-6Rα (Ala^{323}–Val^{362}) was produced by PCR. Its amino acid sequence is GSAAATRAEN EVSTPMQALT TNKDDDNILF RDSANATSLP VQDSSSVAS. The two other linkers were constructed with hybridized oligonucleotides. The 41 amino acids of AGS-41 are GSAAATRGSA GSGGSATGSG SAAGSGDSVA AGSGGGSGSA S. AGS-33 consists of the sequence GSAAATRGSA GSGGSATGSG SAAGSGDSVR RAS. A FLAG tag was added to the C terminus of all fusion proteins using hybridization of an oligonucleotide pair containing *Apa*I, *Xba*I, and *Bam*HI restriction sites and a stop codon. The fusion protein constructs were subcloned into the pIB/v5-his vector (Invitrogen, Groningen, The Netherlands) cut with *Bam*HI and *Hin*dIII (Roche Diagnostic GmbH) to express the protein in insect cells. The integrity of all constructs was verified by DNA sequencing.

Expression in Insect Cells—High 5 (H5) cells cultured in Sf-900II medium (Invitrogen, Paisley, Scotland) were stably transfected with the empty pIB/v5-his vector or vectors containing the fusion protein constructs, using the CellFECTIN method (Invitrogen). Cell supernatants were harvested every 3 days, cleared by centrifugation, and stored at −20 °C until use.

Protein Precipitation—The fusion proteins from cell supernatants were precipitated overnight at 4 °C with IL-6 covalently linked to CNBr-Sepharose (Amersham Biosciences AB, Uppsala, Sweden).

Western Blotting—Proteins were separated by SDS-PAGE, transferred to polyvinylidene difluoride membranes (PALL, Dreieich, Germany), incubated with the antibodies as indicated in the figures, and processed for chemiluminescence detection (Amersham Biosciences AB). Antibodies used for protein detection were as follows: sIL-6Rα (Eurogentec, Philadelphia, PA), Tyr(P) STAT3 (New England Biolabs, Frankfurt, Germany), and STAT3 (Santa Cruz Biotechnology Inc., Santa Cruz, CA).

Purification of the Fusion Proteins—200–500 ml of H5 cell supernatants containing the respective fusion proteins were applied to an IL-6-Sepharose column (2 ml) at 4 °C. After rinsing with phosphate-buffered saline, proteins were eluted with 6 ml of 2 M $MgCl_2$. The eluate was dialyzed against phosphate-buffered saline or cell culture medium, and subsequently the amounts of fusion proteins were measured by ELISA.

Quantification of Fusion Proteins by ELISA—An ELISA procedure was performed as described previously (12), using 0.3 μg/well of FLAG monoclonal antibody (Sigma) for coating and 50 ng/well of biotinylated monoclonal antibody B-T2 (DIACLONE, Besançon, France) as secondary antibody. The standard curve was obtained by 2-fold serial dilutions of sgp130-FLAG expressed in COS-7 cells and calibrated by sgp130 ELISA (12).

Ba/F3 Proliferation Assay—Stably transfected Ba/F3-gp130-IL6R and Ba/F3-gp130-IL11R cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, seeded on 96-well plates (20,000 cells/well), and stimulated with IL-6 (0.9 ng/ml) or trx-IL-11 (5 ng/ml) prepared as described previously (23) in the presence of purified fp stalk-49 (500 ng/ml first concentration) or purified mock-vector-transfected cell supernatant. After 60 h of incubation, metabolically active cells were quantified using a colorimetric assay based on the cell proliferation kit II XTT assay (Roche Diagnostic GmbH).

Induction of Acute-phase Protein Synthesis in HepG2 Cells—α1-Antichymotrypsin synthesized by HepG2 cells was measured by immunoprecipitation of radioactively labeled protein as described previously (12).

RESULTS

Rational Design of a Fusion Protein of sgp130 and sIL-6Rα as a Potential IL-6 Inhibitor—The fusion protein was designed to contain the minimal regions of IL-6Rα and gp130 required for high affinity IL-6 binding. Moreover, the N terminus of mature gp130 should not be affected by the fusion, since it is important for ligand binding (24). Thus, the fusion protein consists of domains D1-D2-D3 of gp130 (including the signal sequence at the N terminus that directs its secretion) followed by a linker and domains D2 and D3 of IL-6Rα (Fig. 1*A*, *upper scheme*). The two receptor fragments have to be connected by the linker in a way that allows the fusion protein to adopt the conformation required for efficient neutralization of IL-6. According to the ternary complex model based on the x-ray structure of viral IL-6 bound to D1–D3 of gp130 (22), the C terminus of gp130-D3 and the N terminus of sIL-6Rα-D2 are separated by at least 8 nm. This distance can be bridged by a peptide linker of about 30–40 amino acids (Fig. 1*A*, *lower part*). The linker should be of high conformational flexibility, of low immunogenicity, and resistant to protease degradation. Three fusion proteins containing different linkers were constructed. Two of them, AGS-33 and AGS-41, are made of flexible Ala-, Gly-, and Ser-rich peptides of 33 and 41 amino acids, respectively. In an extended conformation these linkers span from about 10 (AGS-33) to 12 nm (AGS-41). The third one (fp stalk-49) consists of a short flexible fragment of the extracellular membrane-proximal part of IL-6Rα (25). Besides its flexibility, this linker is expected to be of low immunogenicity, since it is derived from the endogenous IL-6Rα. For technical reasons, a FLAG tag epitope was added at the C termini of all constructs (Fig. 1*A*, *upper scheme*).

Purification and Characterization of Fusion Proteins Produced in Insect Cells—For continuous production of the fusion proteins stably transfected H5 insect cell lines were generated. The fusion proteins were precipitated from cell supernatants with IL-6-Sepharose and analyzed by Western blotting (Fig. 1*B*). The apparent molecular masses of the fusion proteins are 83.5 kDa for fp stalk-49, 69.5 kDa for fp AGS-33, and 72 kDa for fp AGS-41. The substantially higher molecular mass of fp stalk-49 is most likely due to an additional *N*-glycosylation site (Asn-Ala-Thr) introduced with the linker.

We took advantage of the affinity of the fusion proteins to IL-6 for their purification and concentration with IL-6-Sepharose. The insect cell supernatant, the flow-through and the eluate of IL-6 affinity chromatography were analyzed for the presence of fusion protein by Western blotting (Fig. 1*C*). Compared with the supernatant (*sn*, *left lane*), the fusion protein is strongly enriched in the eluate. No fusion protein is detectable in the flow-through fractions. The concentrations of fusion protein in the fractions determined by a newly developed ELISA correlate well with the intensities of the bands in the Western blot (Fig. 1*C*). After dialysis, enriched fp stalk-49 was used for the following studies. Supernatants containing the other fusion

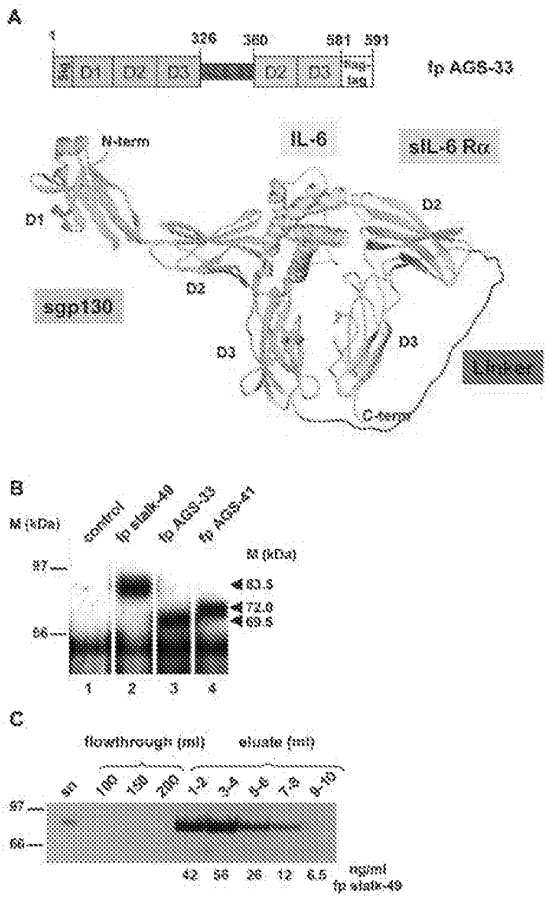

FIG. 1. **Design and characterization of sgp130/sIL-6Rα fusion proteins.** *A*: *upper panel*, schematic representation of the fusion protein fp AGS-33. *Numbers* refer to the amino acid positions in the fusion protein as indicated. *Lower panel*, structural model of IL-6 (*yellow*) bound to a fusion protein of sgp130 D1–D3 (*green*) and sIL-6Rα D2-D3 (*blue*). The gp130 and IL-6 part correspond to the solved structure of viral IL-6 bound to gp130 D1–D3 (22). IL-6Rα D2-D3 is adopted from the recently solved structure of sIL-6Rα (25). The linker (*red*) contains 33 amino acids as in fp AGS-33. Domain D1 of gp130 is involved in dimerization of the depicted ternary complex, leading to a stable hexameric complex (not shown). *B*, 5 ml of supernatants from insect cells stably transfected with expression vectors encoding the fusion proteins or mock-vector (control) were incubated with IL-6-Sepharose. Sepharose-bound proteins were analyzed by immunoblotting using a polyclonal sIL-6Rα antibody. *C*, purification of fp stalk-49 from insect cell supernatant by IL-6 affinity chromatography. 200 ml of supernatant from insect cells expressing fp stalk-49 were loaded onto a 2-ml IL-6-Sepharose column. Bound proteins were eluted with 10 ml of 2 M $MgCl_2$, and 2 ml fractions were collected. The fusion protein from 10 ml of supernatant (*sn*), 10 ml of the fractions of the flow-through after 100, 150, and 200 ml, and from 1.5 ml of the 2-ml fractions of the eluates were precipitated and analyzed by immunoblotting using a polyclonal sIL-6Rα antibody. The indicated concentrations of fp stalk-49 were determined by ELISA.

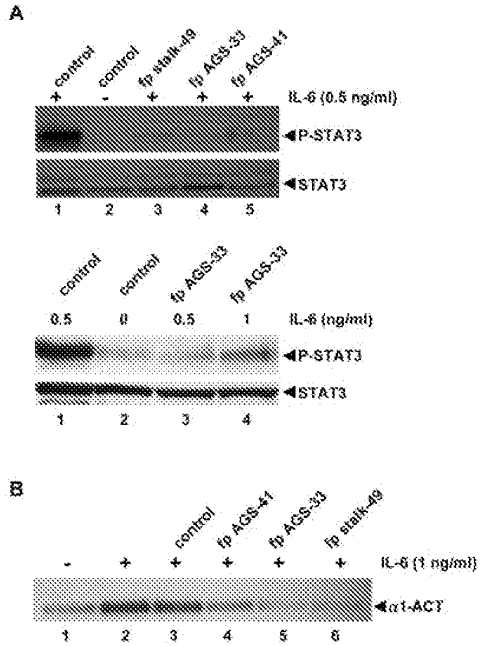

FIG. 2. **IL-6 antagonistic activity of sgp130/sIL-6Rα fusion proteins.** *A*: *upper panel*, Ba/F3-gp130-IL6R cells were incubated with supernatants from insect cells expressing fusion proteins or from mock-transfected insect cells (*control*) and stimulated with 0.5 ng/ml IL-6 (+) for 30 min or left unstimulated (−) as indicated. Activation of STAT3 was analyzed by immunoblotting of cellular lysates. Activation of STAT3 was detected using a polyclonal antibody against tyrosine phosphorylated STAT3. After stripping of the blot, STAT3 loading was controlled using a polyclonal STAT3 antibody. *Lower panel*, Ba/F3-gp130-IL6R cells were incubated with supernatant from insect cells expressing fp AGS-33 or mock-transfected insect cells (*control*) and stimulated with different concentrations of IL-6 for 30 min as indicated. Cellular lysates were analyzed for STAT3 phosphorylation as described above. *B*, HepG2 cells were incubated with 1 ng/ml IL-6 and 30 ng/ml of fusion-proteins or an equivalent volume of purified mock-vector-transfected cell supernatant (*control*) for 18 h and metabolically pulse-labeled with ^{35}S for 3 h. Secreted α1-anti-chymotrypsin (*α1-ACT*) was immunoprecipitated from cell culture supernatants, separated by SDS-PAGE, and analyzed by autoradiography.

proteins and supernatants of mock-transfected insect cells were treated the same way. The latter was used as negative control in the bioassays.

Potent IL-6 Antagonistic Activity of the Fusion Proteins—To test the IL-6 antagonizing activity of the fusion proteins, supernatants of stably transfected insect cells were incubated with IL-6 for 30 min to allow the fusion protein to bind to IL-6. Ba/F3 cells stably transfected with gp130 and IL-6Rα (Ba/F3-gp130-IL6R) were stimulated with the IL-6-treated supernatants. After 30 min, cells were lysed, and STAT3 phosphorylation was analyzed. In the presence of supernatant from mock-transfected insect cells, stimulation of Ba/F3-gp130-IL6R cells with 0.5 ng/ml IL-6 is sufficient to induce prominent tyrosine phosphorylation of STAT3 (Fig. 2*A*, *upper* and *lower panels*, *lanes 1* and *2*). Treatment of Ba/F3-gp130-IL6R cells with IL-6 that was preincubated with supernatants from cells expressing the fusion proteins did not result in significant tyrosine phosphorylation of STAT3 (Fig. 2*A*, *upper panel*, *lanes 3–5*). Thus, all three fusion proteins in the supernatants inhibit IL-6 signaling, since no STAT3 phosphorylation is observed in response to IL-6. A 2-fold higher IL-6 concentration (1 ng/ml) is neutralized only incompletely by the supernatant containing fp AGS-33 (Fig. 2*A*, *lower panel*, *lane 4*).

IL-6 is the major inducer of acute-phase protein synthesis in hepatocytes, but also in hepatoma cell lines such as HepG2. IL-6 stimulation (1 ng/ml) leads to a substantially increased α1-anti-chymotrypsin production by HepG2 cells as shown by immunoprecipitation of metabolically labeled protein (Fig. 2*B*, *lanes 1* and *2*). Purified proteins of control supernatant do not affect α1-anti-chymotrypsin synthesis (Fig. 2*B*, *lane 3*). In the presence of the concentrated fusion proteins (30 ng/ml), α1-anti-chymotrypsin synthesis is reduced to the basal level (Fig. 2*B*, *lanes 4–6*). Thus, all three fusion proteins inhibit IL-6-induced acute-phase protein synthesis.

Specificity of the IL-6-inhibiting sgp130/sIL-6Rα Fusion Proteins—To demonstrate the specificity of the inhibitory fusion

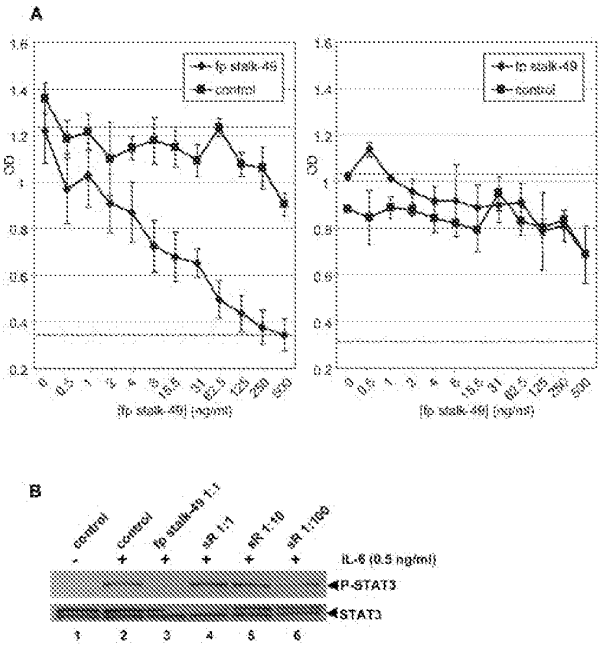

FIG. 3. **Specificity and efficiency of the IL-6-antagonizing sgp130/sIL-6Rα fusion proteins.** *A*, Ba/F3-gp130-IL6R (*left panel*) or Ba/F3-gp130-IL11R cells (*right panel*) were incubated with a constant amount of IL-6 (0.9 ng/ml) or trx-IL-11 (5 ng/ml), respectively, and serial 2-fold dilutions of inhibitors starting with a concentration of 500 ng/ml. Equivalent volumes of purified mock-vector-transfected cell supernatant were used as a control. After 60 h of incubation, viable cells were quantified using the colorimetric XTT assay (Roche Diagnostic GmbH). *Dashed lines* and *dotted lines* correspond to proliferation in the absence of cytokine or inhibitor, respectively, derived from the standard curves (not shown). Mean values from three independent experiments are shown with S.D. values. *B*, Ba/F3-gp130-IL6R cells were stimulated for 20 min with 0.5 ng/ml IL-6, which was preincubated for 15 min at 4 °C with 1.5 ng/ml fp stalk-49 or the equivalent volume of purified mock-vector-transfected cell supernatant (*control*) or with increasing amounts of soluble receptors (*sR*): 1 ng/ml sIL-6Rα and 2 ng/ml sgp130 (*lane 4*), 10 ng/ml sIL-6Rα and 20 ng/ml sgp130 (*lane 5*); and 100 ng/ml sIL-6Rα and 200 ng/ml sgp130 (*lane 6*). Activation of STAT3 was analyzed by immunoblotting of cellular lysates. STAT3 tyrosine phosphorylation was detected as described in legend to Fig. 2A. sIL-6Rα and sgp130 were prepared as described previously (12, 33).

proteins, we compared the proliferation of Ba/F3 cells stably transfected with gp130 and IL-6Rα or gp130 and IL-11Rα (Ba/F3-gp130-IL11R) in response to 0.9 ng/ml IL-6 or 5 ng/ml IL-11, required for 50 or 80% of maximal cell proliferation, respectively. Trx-IL-11 is a fusion protein of thioredoxin and IL-11 exhibiting IL-11 activity indistinguishable from IL-11 wild type (23). The proliferation of Ba/F3-gp130-IL6R cells treated with a constant amount of IL-6 is inhibited by the fusion protein fp stalk-49 in a concentration-dependent manner. Purified control supernatant had no significant effect (Fig. 3*A*, *left diagram*). At a fusion protein concentration of 250–500 ng/ml, cell proliferation is completely abrogated. Proliferation of Ba/F3-gp130-IL11R cells in response to trx-IL-11, however, is not significantly disturbed by fp stalk-49 (Fig. 3*A*, *right diagram*). Thus fp stalk-49 specifically inhibits IL-6, but not IL-11 responses. Similar observations were made for the remaining fusion proteins (data not shown).

Inhibitory Activity of the Fusion Proteins Compared with Separately Expressed sgp130 and sIL-6Rα—Next, we proved that the appropriate fusion of the ligand-binding domains of gp130 and IL-6Rα leads to a more potent inhibitor than sgp130 and sIL-6Rα separately expressed in insect cells. The inhibition of STAT3 phosphorylation in Ba/F3-gp130-IL6R cells induced by 0.5 ng/ml IL-6 achieved with 1.5 ng/ml fp stalk-49 was compared with inhibition by the combination of sgp130 and sIL-6Rα (Fig. 3*B*). The approximately equimolar ratio of IL-6 and fp stalk-49 is sufficient for almost full suppression of IL-6 induced STAT3 activation (*lanes 1–3*). In contrast to this extremely high antagonistic potency of the fusion protein, a molar ratio of 1:100 of IL-6 and the combination of sIL-6Rα and sgp130 is required to achieve inhibitory activity (*lanes 4–6*). We conclude that the fusion proteins are of ~100-fold increased inhibitory activity compared with the separate soluble receptor proteins.

DISCUSSION

In this study we present a highly potent IL-6 inhibitor based on the ligand-binding domains of the IL-6 receptor subunits IL-6Rα and gp130. Many of the existing IL-6 receptor antagonists are IL-6 mutants of binding sites to gp130 (sites II and III). They block IL-6Rα by binding *via* the intact site I but do not recruit gp130 (26, 27). Since the interaction of IL-6 with IL-6Rα is of low affinity, so called superantagonists were created by mutating the site I of these antagonists to strengthen α-receptor binding. Although the superantagonists perform better, they still have to be applied in a large excess to IL-6 (27). Furthermore, due to the many mutations the proteins are highly immunogenic (28). Neutralizing IL-6 or IL-6Rα antibodies have also been used as IL-6 inhibitors. They were tested in clinical trials for the treatment of rheumatoid arthritis (3) or AIDS-associated Kaposi's sarcoma (29) but turned out to be of rather low efficiency. Very recently, potent low molecular mass IL-6 receptor antagonists were described for the first time (30, 31). These antagonists have to be applied in the micromolar range to inhibit picomolar amounts of IL-6.

A new generation of cytokine antagonists is based on soluble receptor fragments that bind the ligand with high affinity and specificity. In the case of IL-6, two receptor subunits are required for high affinity binding, IL-6Rα and gp130. Moreover, the complex of IL-6 and sIL-6Rα acts agonistically on cells expressing gp130 (10). Conversely, sIL-6Rα supports neutralization of IL-6 by sgp130 due to formation of a soluble high affinity ternary complex (12). The new IL-6 receptor antagonist presented in this study stems from the idea that appropriate fusion of the ligand-binding domains of IL-6Rα and gp130 should result in a superior antagonist that neutralizes IL-6 with highest affinity and specificity. In the present study three different linkers were used to connect the ligand-binding domains of gp130 and IL-6Rα. It turned out that the fusion proteins exhibit similar inhibitory activities, indicating that the estimation of the required linker length has been correct and appropriate peptide linkers were chosen.

All three fusion proteins bind IL-6 as shown by precipitation with IL-6-Sepharose. The fusion protein present in the insect cell supernatant is sufficient to completely antagonize the activity of 0.5 ng/ml (25 pM) IL-6 in the short term STAT3 phosphorylation assay using transfected Ba/F3 cells (Fig. 2*A*). Since the concentrations of the fusion proteins in the insect cell supernatants are in the range of 1–2 ng/ml (15–30 pM), this points to an inhibitory activity at a molar ratio between agonist and antagonist of 1:1.

In a long term assay such as induction of acute-phase protein synthesis in HepG2 cells, the activity of 1 ng/ml (50 pM) IL-6 was totally blocked by the addition of inhibitory fusion protein at a nearly 10-fold molar excess (450 pM). In the Ba/F3 proliferation assay with fp stalk-49, we determined an IC_{50} of 6 ng/ml (90 pM) for the inhibition of 0.9 ng/ml IL-6 (45 pM). Thus, in long term assays and therefore also for studies of the inhibitory activity of the fusion proteins *in vivo*, an about 10-fold molar excess of fusion protein over IL-6 should be applied.

Besides their inhibitory activity the specificity of the fusion

proteins is an important feature to assess their potential value for anti-cytokine therapies. IL-11 most closely resembles IL-6 because it also signals *via* gp130 homodimers but binds to a different α-receptor, namely IL-11Rα. In the Ba/F3-proliferation assay, amounts of fusion proteins that significantly inhibit IL-6 activity had no effect on IL-11 induced proliferation (data shown only for fp stalk-49). Therefore, at the concentrations used in our assays each of the three fusion proteins is a potent and specific inhibitor of IL-6 activity.

The superior activity of the fused ligand-binding domains of gp130 and IL-6Rα compared with the separate soluble receptors sgp130 and sIL-6Rα is probably the most important issue left to be proven to confirm the value of our concept. IL-6-induced STAT3 phosphorylation in Ba/F3 cells is inhibited by the presence of equimolar amounts of fusion protein. To achieve a similar inhibition an at least 100-fold molar excess of sgp130 and sIL-6Rα has to be applied. This intriguing result clearly demonstrates the extraordinary high inhibitory activity of the fusion protein. What is the explanation for this finding? In the above assay, a low amount of IL-6 that is in the range of pathophysiological IL-6 concentrations (500 pg/ml) was applied. When the separate soluble receptors were used, IL-6 first binds to the sIL-6Rα. This interaction is of low affinity, and therefore the complex of IL-6 and sIL-6Rα might dissociate before it encounters sgp130. In the fusion protein, the initial complex of IL-6 bound to domains D2 and D3 of IL-6Rα can be immediately trapped by the covalently linked ligand-binding domains of gp130 before dissociation occurs.

Our inhibitor strategy is also applicable to other cytokines that signal *via* heteromeric receptor complexes. Indeed, in a recent publication Economides *et al.* (32) used a similar approach to create so called "cytokine traps" as highly potent inhibitors for IL-1, IL-4, and IL-6. In their study the complete ectodomains of the respective receptor subunits including the regions dispensable for ligand binding were fused to the Fc part of human IgG. This results in dimerization of the receptor chains by disulfide bond formation of the Fc parts. In the case of the IL-6 inhibitor, this leads, besides the desired sgp130-Fc/sIL-6Rα-Fc heterodimers, to the formation of gp130-Fc/gp130-Fc and sIL-6Rα-Fc/sIL-6Rα-Fc homodimers. As a consequence, before application, the heterodimer must be separated from the homodimers. On the XG-1 myeloma cells an IC_{50} of 50 pM sgp130-Fc/sIL-6Rα-Fc was determined for the neutralization of 2.5 pM (0.05 ng/ml) IL-6 (32). On our Ba/F3-gp130-IL6R cells treated with 45 pM IL-6, the IC_{50} of fp stalk-49 is 90 pM. If one takes into account the molar ratio between the IL-6 concentration in the two different proliferation assays and the IC_{50} of the respective inhibitor, it turns out that fp stalk-49 is about 10-fold more potent than sgp130-Fc/sIL-6Rα-Fc. These findings suggest that the more structure-based approach presented in our study confirms the validity of the basic concept and furthermore leads to optimized inhibitory fusion proteins.

We conclude that appropriate fusion of the ligand-binding domains of soluble receptor proteins leads to cytokine inhibitors of extraordinary activity which might be of considerable therapeutical value for the development of new anti-cytokine therapies.

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A Fusion Protein of the gp130 and Interleukin-6Rα Ligand-binding Domains Acts as a Potent Interleukin-6 Inhibitor

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Crystal structure of a cytokine-binding region of gp130

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The structure of the cytokine-binding homology region of the cell surface receptor gp130 has been determined by X-ray crystallography at 2.0 Å resolution. The β sandwich structure of the two domains conforms to the topology of the cytokine receptor superfamily. This first structure of an uncomplexed receptor exhibits a similar L-shaped quaternary structure to that of ligand-bound family members and suggests a limited flexibility in relative domain orientation of some 3°. The putative ligand-binding loops are relatively rigid, with a phenylalanine side chain similarly positioned to exposed aromatic residues implicated in ligand binding for other such receptors. The positioning and structure of the N-terminal portion of the polypeptide chain have implications for the structure and function of cytokine receptors, such as gp130, which contain an additional N-terminal immunoglobulin-like domain.
Keywords: cytokine receptor/gp130/multiple anomalous diffraction/X-ray crystallography

Introduction

Cytokines, generally in the form of secreted molecules, mediate intercellular signalling by high affinity interaction (K_D ~10^{-10} M) with the extracellular regions of specific cell surface receptors. This promotes receptor oligomerization which, in turn, triggers intracellular signalling cascades within the target cell. The ability of a given cytokine to elicit biological responses in a target cell is therefore dictated by the specificity of interaction between ligand and receptor.

Gp130 is a transmembrane receptor which is required for signal transduction by a set of cytokines, the gp130 family, which have many significant biological functions of potential therapeutic interest (reviewed in Kishimoto *et al.*, 1995). Gp130-mediated signalling has been implicated in the regulation of a wide variety of adult tissue systems, including haemopoesis, nervous system, bone, heart, adipose tissue, testes, liver and muscle (reviewed in Kishimoto *et al.*, 1995). Targeted inactivation of the gp130 gene results in a complex pre-natal lethal phenotype including defects in cardiac and haematological function (Yoshida *et al.*, 1996). In addition, chronic activation of gp130 signalling in a transgenic mouse model results in cardiac hypertrophy (Hirota *et al.*, 1995). The gp130 family of ligands currently comprises interleukin-6 (IL-6), IL-11, herpes virus IL-6 (HSVIL-6), leukaemia inhibitory factor (LIF), oncostatin (OSM), cardiotrophin (CT-1) and ciliary neurotrophic factor (CNTF). The three-dimensional structures of three members of the gp130 family, LIF (Robinson *et al.*, 1994), CNTF (McDonald *et al.*, 1995) and IL-6 (Somers *et al.*, 1997; Xu *et al.*, 1997) have been defined by crystallographic or solution NMR techniques. This reveals that these cytokines share a common topology, being composed of four regions of α helix (helices A–D) linked by polypeptide loops in the 'up-up-down-down' conformation typical of the 'long chain' family of cytokines (Boulton *et al.*, 1994).

The signalling functions of gp130 are initiated by the ligand-mediated formation of oligomeric complexes with other specific partner receptors. Gp130 initially was cloned as an essential transmembrane component for signalling mediated by IL-6 (Hibi *et al.*, 1990). This occurs via dimerization of gp130 (Murakami *et al.*, 1993) following the formation of a hexameric complex containing two molecules of gp130, two molecules of IL-6 and two molecules of a soluble specific IL-6 receptor (Ward *et al.*, 1994; Paonessa *et al.*, 1995). A similar mechanism pertains to the case of IL-11, where homodimerization of gp130, and subsequent execution of signalling functions, is brought about by association with a complex of IL-11 and specific IL-11 receptors (Hilton *et al.*, 1994; Karow *et al.*, 1996).

Gp130 was also cloned as a receptor required for signalling mediated by cytokines which associate with a second transmembrane receptor of the cytokine type–LIF-R (Gearing *et al.*, 1991). These include OSM (Gearing *et al.*, 1992; Liu *et al.*, 1992), LIF and CNTF (Ip *et al.*, 1992), and CT-1 (Pennica *et al.*, 1995). In this case, signal transduction is initiated by ligand-mediated heterodimerization of gp130 and LIF-R (facilitated, in the case of CNTF, by association with a third non-signalling receptor component CNTF-R). Recently it has been discovered that OSM can also mediate signalling by heterodimerization of gp130 with a novel transmembrane signalling receptor of the cytokine type, OSM-R (Mosley *et al.*, 1996). The intracellular signalling pathways activated by ligand-mediated homo- or heterodimerization of gp130 include activation of the receptor-associated JAK/Tyk tyrosine kinases (Boulton *et al.*, 1994; Stahl *et al.*, 1994), the STAT family of transcription factors (Stahl *et al.*, 1995) and src-family tyrosine kinase pathways (Ernst *et al.*, 1994).

The sequence of the extracellular ligand-binding region of gp130 reveals that it is a member of the 'cytokine' superfamily of receptors characterized by a canonical cytokine-binding homology region (CHR) containing the

Table I. gp130-CHR data collection statistics

	Wild-type	Seλ_1	Seλ_2	Seλ_3	Seλ_4	Seλ_5
Wavelength (Å)	1.030	9790	0.9791	0.9535	0.9793	0.9789
Unique	48 189	20 549	20 280	22 400	20 264	19 303
$<I/\sigma(I)>$	19.4 (5.0)	23.2	27.2	24.8	18.8	28.6
R_{merge} (%)	5.8 (20.6)	5.1	4.3	4.2	4.2	5.0
Resolution (Å)	30–2.0	30–2.55	30–2.55	30–2.50	30–2.55	30–2.55
Completeness (%)	98.8 (96.6)	93.6	93.5	92	93.5	89.4

$R_{merge} = \Sigma|I - <I>| / \Sigma<I>$.
Values in parentheses correspond to the highest resolution shell (2.07–2.00 Å).

'WSXWS' motif, a proline-rich 'hinge' region and a characteristic spacing of cysteine residues (reviewed in Cosman, 1993). In addition, the extracellular region of gp130 contains an N-terminal module predicted to adopt a seven-stranded immunoglobulin-like conformation and, C-terminal to the CHR, three fibronectin type III (FN III) domains. Deletion studies have revealed that the gp130-CHR is sufficient for interaction with ligand (Horsten *et al.*, 1995). Mutation studies of both IL-6 (Paonessa *et al.*, 1995) and LIF (Hudson *et al.*, 1996) have shown that this interaction involves topologically analogous receptor recognition epitopes (site II) in both ligands. A second, physically discrete, gp130 ligand recognition epitope (site III) has also been described for the interaction with IL-6 (Paonessa *et al.*, 1995). This interaction requires regions of gp130 outside the CHR (Simpson *et al.*, 1997; D.Staunton, K.R.Hudson and J.K.Heath, unpublished observations). The function of the CHR of gp130 is therefore the association with partner ligands (alone or complexed with receptor) via their site II recognition epitopes.

Crystal structures are available for four cytokine receptors containing a CHR; the growth hormone receptor (GHR; De Vos *et al.*, 1992), the prolactin receptor (PRLR; Somers *et al.*, 1994), the erythropoietin receptor (EPOR; Livnah *et al.*, 1996) and the interferon-γ receptor (IFNγ-R; Walter *et al.*, 1995). These four prototypes undergo an exclusively homodimerization mode of action with a restricted range of ligands; little is known currently of the detailed structural features of receptors which undergo heterodimerization in the presence of ligand or interact with multiple ligand and receptor partners.

We report here the high resolution crystal structure of the cytokine-binding homology region of gp130. This structure provides the first detailed three-dimensional information for a receptor component crucial to the signalling complexes of a large family of growth factors (IL-6, IL-11, LIF, CNTF, CT-1 and OSM) allowing assessment of the molecular basis of specific recognition and ligand engagement.

Results and discussion

Expression and structure determination

A soluble form of the gp130 cytokine-binding homology region (gp130-CHR) was expressed in *Escherichia coli* as a maltose-binding protein (MBP) fusion (D.Staunton, K.R.Hudson and J.K.Heath, in preparation). The final purified protein comprised four residues from the fusion linker, residues 100–303 of human gp130 (the CHR) and a further 14 residues corresponding to a three-alanine linker and c-myc tag. Surface plasmon resonance studies confirmed that this recombinant form of gp130-CHR binds OSM with an affinity (K_d ~6.5×10^{-8} M) equivalent to the complete gp130 extracellular domain expressed in eukaryotic cells (D.Staunton, K.R.Hudson and J.K.Heath, in preparation). Gp130-CHR therefore retains structural features required for ligand engagement via site II.

Crystallization trials yielded highly ordered crystals of space group $C222_1$ (unit cell dimensions a = 84.5 Å, b = 132.3 Å, c = 121.9 Å) which contained two gp130-CHR molecules per crystallographic asymmetric unit. The non-crystallographic symmetry does not reveal any possible mode of receptor dimerization. The structure was determined by multiple anomalous dispersion (MAD) phasing techniques using X-ray diffraction data collected on BM14 at the European Synchrotron Radiation Facility (ESRF) from crystals of a selenomethionyl form of the protein. The structure has been refined to a crystallographic R-value of 21.5% for all data between 30 and 2.0 Å resolution. Crystallographic statistics are reported in Tables I and II. All 204 residues of the CHR are well ordered for one of the two copies in the crystallographic asymmetric unit, but residue 100 at the N-terminus and residues 212–213 of a loop region are disordered in the second copy; the final model also includes certain of the residues which derive from the expression construct (Figure 1A, see Materials and methods). Domain-wise superpositions of the two molecules in the crystallographic asymmetric unit show essentially identical structures (r.m.s. deviation for equivalent C_α atoms between 90 residues of the CHR N-terminal domain is 0.44 Å and between 99 residues of the CHR C-terminal domain is 0.32 Å). The following text focuses exclusively on the structure of the human gp130-CHR and refers to these residues by the intact gp130 numbering, 100–303.

Structure description and comparison with other CHR structures

As anticipated from sequence analysis, the topology of gp130-CHR is similar to those of the three other class 1 receptors of the cytokine superfamily (Cosman, 1993) for which structures have been determined (hGHR, de Vos *et al.*, 1992; hPRLR, Somers *et al.*, 1994; EPOR, Livnah *et al.*, 1996). The CHR comprises two specialized FN III domains. The basic structural scaffold for each domain consists of a β sandwich primarily formed from a three-strand (A, B, E) and four-strand (C, C′, F, G) β sheet. These domains are connected by a short 3_{10} helix and are oriented such that the whole molecule has an approximate L shape (Figure 1).

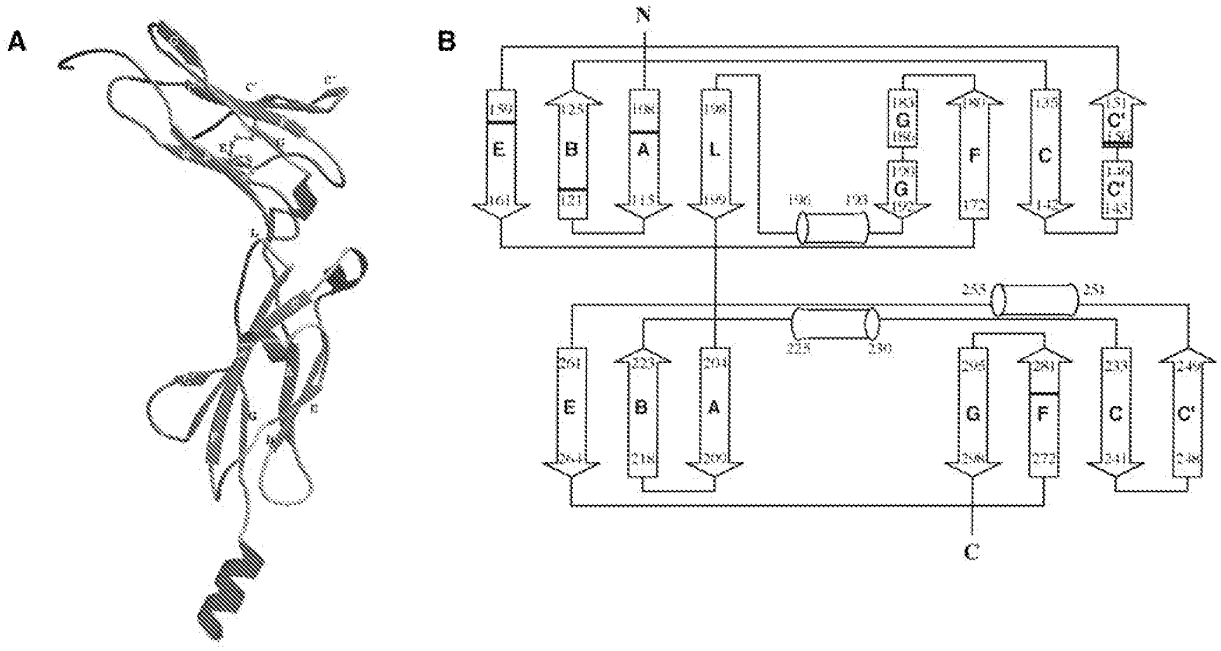

Fig. 1. The structure of gp130-CHR. (**A**) Ribbon representation of the structure of gp130-CHR. Helical segments are shown in red and β strands in green. The full crystal structure is illustrated which includes, in addition to the structure of residues 100–303 of gp130, an extra three N-terminal residues and eight C-terminal residues which derive from the expression construct. The C-terminal α helix results from these latter residues. (**B**) Topology diagram of the two domains of gp130-CHR. Helices are represented by cylinders and β strands by arrows. The positions of the five cysteine residues are marked by black bars. (A) and all components of Figures 2, 3 and 5 were drawn using programs MOLSCRIPT (Kraulis, 1991), with modifications by R.Esnouf (Esnouf, 1997), and RASTER3D (Merrit and Murphy, 1994).

Gp130-CHR D1

The N-terminal domain (D1; residues 103–192) has the standard arrangement of A, B, C, C′, E, F and G β strands (Figures 1 and 2). One notable feature, unique to gp130-CHR D1, is the division of strand G into two approximately equal portions. This is the result of a β bulge and, as discussed below, shows similarity to the WSXWS motif of the second domain. In common with other examples of this fold, gp130-CHR D1 contains two disulfides which link the A and B strands (Cys112–Cys122) and the C′ and E strands (Cys150–Cys160). The lengths of the interstrand loops are similar to those in the EPOR D1 (with the exception of the shorter region between strand C′ and E, and a longer FG loop) and show little significant increase in flexibility relative to the core of the domain (as judged from crystallographic *B*-factors).

Given this general level of topological equivalence, it is somewhat surprising to find that structural superpositions with other CHR D1 structures (Figure 2A) show relatively poor agreement within the core framework (for example, 1.0 Å r.m.s. deviation for 53 structurally equivalent Cα atoms with EPOR D1). As illustrated in Figure 2B, this discrepancy arises from the distinctive angle of β strands C, F and G in the upper half of the GFCC′ sheet. A superposition on gp130-CHR D2 shows a more extensive match over these main secondary structure elements (1.2 Å r.m.s. deviation for 64 structurally equivalent Cα atoms). The key feature, common to both gp130-CHR D1 and D2, is the region of extended polypeptide chain (residues 102–107 of D1, residues 198–204 of D2) which packs tightly against the edge of the β sandwich between strands B and G before starting strand A at Asn109 in D1 and Asn205 in D2. The tight packing of this part of the polypeptide chain against the core of the β sandwich is mediated by the insertion of proline residues Pro103 and Pro107 in D1, and Pro200 and Pro203 in D2. The incorporation of this feature necessitates the shift in orientation of the upper part of the GFC sheet. In gp130-CHR D1, this is achieved through the distinctive β bulge in strand G at residues 187–189 stabilized by the hydrogen bonding of the Ser187 hydroxyl to the main-chain nitrogen of Val176 in strand F (Figure 2C). An identical function is performed by the two serine residues in the canonical WSXWS motif of CHR D2 structures, with the superposition of the gp130-CHR D1 and D2 domains indicating that Ser187 and Ser292 are structurally equivalent. None of the other class 1 CHR D1 structures contain the equivalent length of polypeptide tightly clamped between the B and G β strands or the β bulge in strand G.

Gp130-CHR D2

The C-terminal domain (D2; residues 200–300) conforms to the standard A,B,E and GFCC′ β sheet arrangement. Of the two domains of the CHR, the second appears to be generally the more structurally conserved within the cytokine receptor family. Structural superpositions indicate closest similarity to the EPOR domain (1.05 Å r.m.s. deviation for 81 structurally equivalent Cα atoms). This arises primarily from the shorter length of the β strands in these two molecules compared with those in other members of the superfamily. The only secondary structure element to not correspond closely in position between the gp130-CHR and EPOR D2 structures is the C′ strand. In common with the other class-1 CHR D2 structures, the

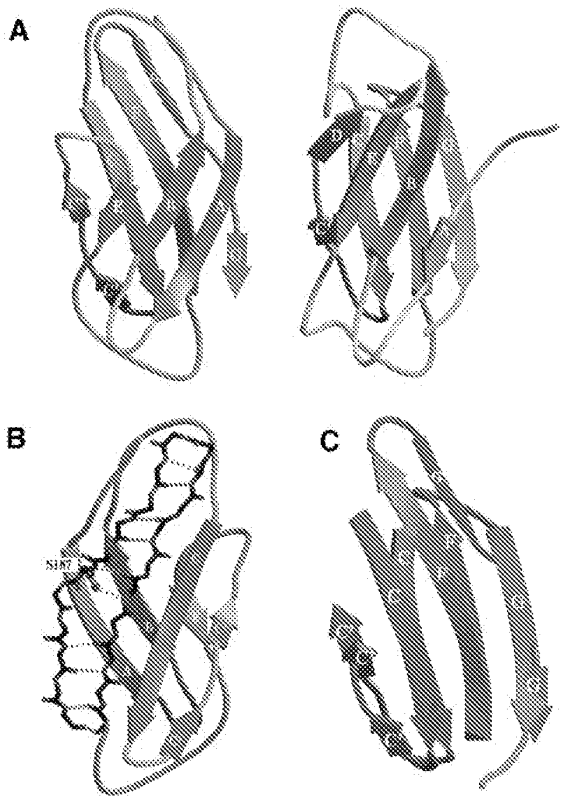

Fig. 2. Gp130-CHR domain 1. (**A**) Comparison of D1 of gp130 (green) and D1 of EPOR (brown). The C′ and G strands are broken into two portions in gp130, and there is no D strand. (**B**) The β bulge in strand G. The main-chain atoms of β strands F and G are shown in stick representation, Ser187 is shown in ball and stick representation, and hydrogen bonds are denoted by broken lines. (**C**) The distinctive C′,C,F,G sheet in gp130. The C′,C,F,G strands are shown for gp130 (green) and EPOR (brown) positioned on the basis of a whole domain superposition (performed using the program SHP; Stuart *et al.*, 1979) to illustrate the novel nature of the top half of the gp130 β sheet.

interstrand loops in gp130-CHR D2 show relatively limited mobility (as judged from crystallographic *B*-factors), with the exception of the AB loop. This loop is stabilized by a crystal contact in one copy of the gp130-CHR but in the other copy the AB loop is exposed to solvent and is disordered in the electron density map (residues 212 and 213). The domain contains one free cysteine residue (Cys279) which is buried within the core of the β sandwich. This precludes it from involvement in disulfide-linked homodimerization of gp130 during IL-6-related cytokine signalling (Murakami *et al.*, 1993).

The WSXWSX sequence (gp130 residues 288–293), a defining feature of this receptor superfamily, is situated in the N-terminal portion of strand G (Figure 3A and B) and has an essentially identical double β bulge structure to that of the homologous region in EPOR. The two successive β bulges are stabilized by hydrogen bonds from the hydroxyls of Ser289 and Ser292 to the main-chain nitrogens of Cys279 and Ile277 respectively. As in the other class-1 CHR D2 structures, the side chains of Trp288 and Trp291 participate in an extended π-cation system, stacking between the side chains of strand F residues Arg276, Arg278 and Met280. This feature is considerably more extended in gp130-CHR D2 than in EPOR D2, however, since it also involves side chains from residues Arg240 and Trp247 in strand C and at the start of strand C′ respectively. The absence of these latter interactions in EPOR may underlie the difference in the position of its C′ strand relative to that in gp130-CHR D2. The extended π-cation system in gp130 is most closely matched by that in PRLR.

The interdomain region and relative domain orientation

As observed for the other superfamily members, residues of the interdomain linker region (gp130 residues 193–197) form a 3_{10} helix. Additionally, residues 198 and 199 in gp130-CHR form a short β strand. This novel structural element hydrogen-bonds to both strand A of D1 and the WSXWS region at the N-terminus of strand G in D2, providing an extra constraint on the relative orientation and positioning of the two domains (Figure 3C). The resultant juxtaposition of D1 and D2 produces a tight interdomain interface which, excluding the contribution of the linker, buries ~350 Å^2 of solvent-accessible surface. This is contributed mainly by residues in strand A and the EF loop of D1 (I113, E116 and Y168) and residues from the BC loop and strand G of D2 (I227, V230, I231 and Y287). Superposition of the two copies of gp130-CHR in the crystallographic asymmetric unit reveals a 3° difference in the relative orientation of their domains. This appears to originate from very slight changes in the main-chain torsion angles for linker residues V198–K199. The structural constraints imposed by the linker 3_{10} helix and β strand plus the hydrophobic, close-packed nature of the interface, argues against any more substantial degree of interdomain orientational freedom than the observed 3° range.

The overall shape of the molecule can be quantified in terms of a tilt angle (defined as the angle between the long axes, running approximately parallel to the β strands, in the two domains; Bork *et al.*, 1996). With a tilt angle of 78°, the relative domain orientation in gp130-CHR corresponds most closely to the general 'L-shaped' (~90° tilt angle) arrangement characteristic of the other class 1 members of the superfamily (hGHR, hPRLR and EPOR) rather than the more upright (~50° tilt angle) arrangement of the more distantly related class 2 members IFNγ-R and TF or the more extreme 120° or so of tilt very recently observed in the natural killer inhibitory receptor (Fan *et al.*, 1997). A detailed comparison (combining the effect of tilt angle and twist in the orientation of D1 relative to the D2 axis) reveals that the precise interdomain orientation in gp130-CHR differs by some 23° from that of the most closely related quaternary structure, that of EPOR.

Sequence comparisons with other species

The human gp130-CHR sequence employed in this study was aligned with the homologous regions from mouse, rat and *Xenopus* gp130 (Figure 4). This reveals that 74/204 (36%) of residues in this region are conserved amongst all versions of the gp130-CHR. It is notable that the majority (64) of these shared residues are in a relatively buried location in the human gp130-CHR structure. This indicates that these conserved residues most probably play a role in determining the structural framework of gp130

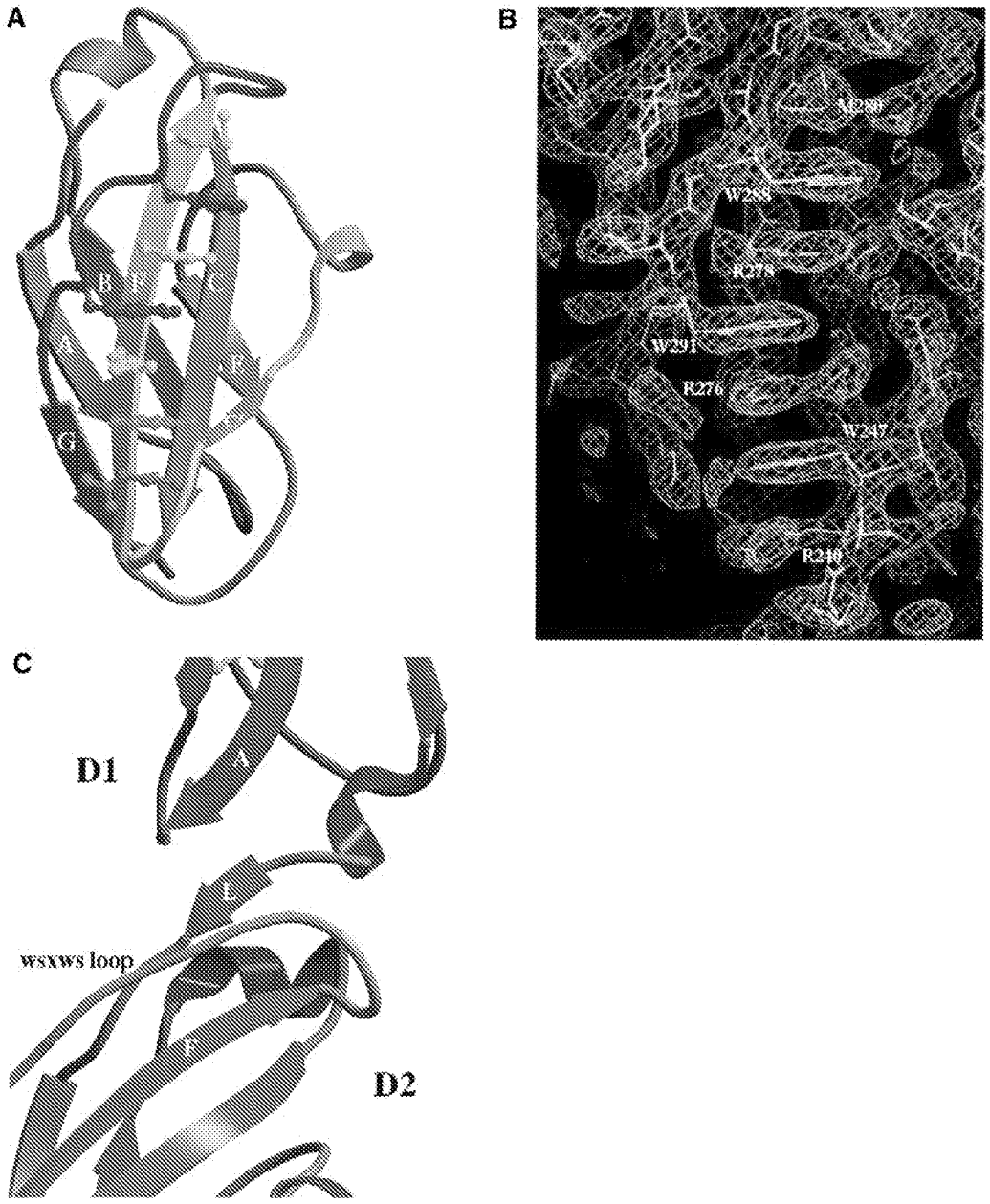

Fig. 3. Gp130-CHR domain 2 and interdomain linker. (**A**) Schematic diagram of the WSXWS box. The side chains of residues contributing to this structural feature are colour coded according to the secondary structure element from which they originate. (**B**) Atomic coordinates and electron density map for the WSXWS box. The refined coordinates are displayed with the original 2.9 Å resolution electron density map calculated using MAD phases followed by density modification (program DM, see Materials and methods). The map is contoured at 1σ in program O. (**C**) Schematic diagram of the D1–D2 linker region. Strand L in the linker region hydrogen-bonds to strand A in DL and the WSXWS box region of the polypeptide chain prior to the start of strand G in D2.

rather than ligand recognition. It follows that the putative ligand recognition epitopes of gp130 may exhibit variation between species.

Implications for ligand recognition

The topological similarity of gp130 to hGHR and hPRLR, systems for which ligand binding has been structurally and functionally well characterized (Cunningham and Wells, 1989; reviewed in Sprang and Bazan, 1993; Wells *et al.*, 1993), permits the identification of candidate structural features of gp130 that mediate ligand recognition via site II. The cognate site II gp130 recognition epitopes have been defined for two ligands, LIF (Hudson *et al.*, 1996) and IL-6 (Savino *et al.*, 1994). In both cases, these consist of a small number (4–6) of solvent-exposed residues located in the adjacent helices A and C of the ligand. This suggests that the site II ligand recognition site of gp130 will also be formed from relatively few

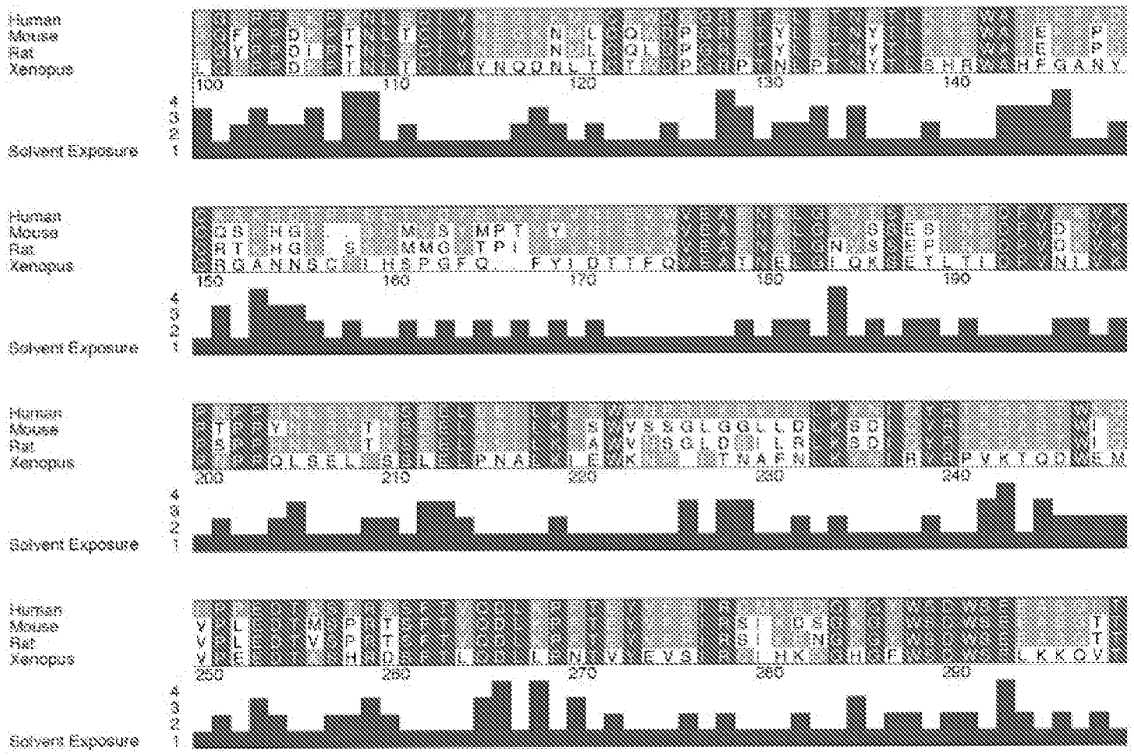

Fig. 4. Sequence alignment and solvent accessibility of gp130 sequences. The CHR region of human (Swissprot accession No. P40189, Hibi *et al.*, 1990), murine (Q00560, Saito *et al.*, 1992), rat (P40190, Wang *et al.*, 1992) and *Xenopus* (K.Chien, A.Grace and J.Chen, personal communication) gp130 sequences were aligned using the progressive pairwise algorithm of Feng and Doolittle (1987) implemented in the Pileup programme of the GCG package followed by minor manual editing. Solvent exposure scores were calculated using DSSP implemented in the program Turbo-6 (Roussel and Cambillau, 1989). Scores 0–50 were assigned a value of 1, 51–100:2, 101–150:3 and 151–200:4. Conserved residues are coloured in orange and residues which are identical to human gp130 are coloured in red. The figure was generated using the programme Alscript (Barton, 1993).

solvent-exposed residues forming a complementary binding site.

The hGH–hGHR complex (De Vos *et al.*, 1992) and the related hGH–hPRLR complex (Somers *et al.*, 1994) reveal that recognition of the ligand via site II involves solvent-exposed residues located in three loops linking the main β strand elements. The first of these is a prominent aromatic residue located in the loop between strands E and F of D1 (Trp104 in both hGHR and hPRLR). In the human gp130-CHR structure, the analogous EF loop contains a similar prominent, solvent-exposed residue Phe169 (Figure 5). Sequence alignment of gp130 sequences (Figure 4) suggests that the analogous residue is present in rat gp130 but is replaced by the conservative substitution of a tyrosine residue in the mouse and *Xenopus* proteins. Sequence alignment of the equivalent region in other receptors for members of the long chain cytokine family reveals that a non-polar residue (Phe, Tyr or Trp) in the predicted EF loop of D1 is a common feature (data not shown).

The other two potential binding sites are located in D2 in the form of the loops linking strands BC (gp130 residues 226–230) and FG (gp130 residues 281–285). A non-conservative substitution mutant V230D, which is located in the BC loop, results in loss of affinity for IL-6–IL-6R (Horsten *et al.*, 1997). V230 is, however, relatively buried in the three-dimensional structure (DSSP score of relative exposure 23%), and the effect of this mutation on ligand recognition may, therefore, be indirect. The FG loop has also been implicated recently in the recognition of granulocyte colony-stimulating factor (GCSF) by the D2 domain of the GCSFR CHR (Yamasaki *et al.*, 1997). In addition, non-conservative substitution mutants of two exposed residues (G286W and K285E) in this region of gp130 result in loss of affinity for IL-6–IL-6R (Horsten *et al.*, 1997), suggesting that the FG loop does indeed play a significant role in ligand recognition. The equivalent regions of gp130 proteins from other species exhibit conservation, but not identity, of residues in these regions.

A key feature of gp130 is its ability to interact with a range of ligands in the context of a number of other partner receptors. In cases such as LIF and OSM (Hudson *et al.*, 1996), the ligand is able to interact with gp130 with high affinity on its own. In other cases such as IL-6 and IL-11, high affinity interaction between gp130 and the ligand requires that ligand is associated with a partner receptor (IL-6R and IL-11R respectively). This suggests that there may exist additional sites on gp130 which are involved in interaction with partner receptors. Mutagenesis of the IL-6R (Yawata *et al.*, 1993) and the IL-11R (M.A.Hall, P.Bilinski, A.Gossler and J.K.Heath, unpublished observations) have revealed that specific non-conservative substitutions of residues in the membrane-proximal region of the predicted D2 in these receptors can block the ability of the ligand–receptor complex to interact with gp130. Inspection of the dimeric hGHR

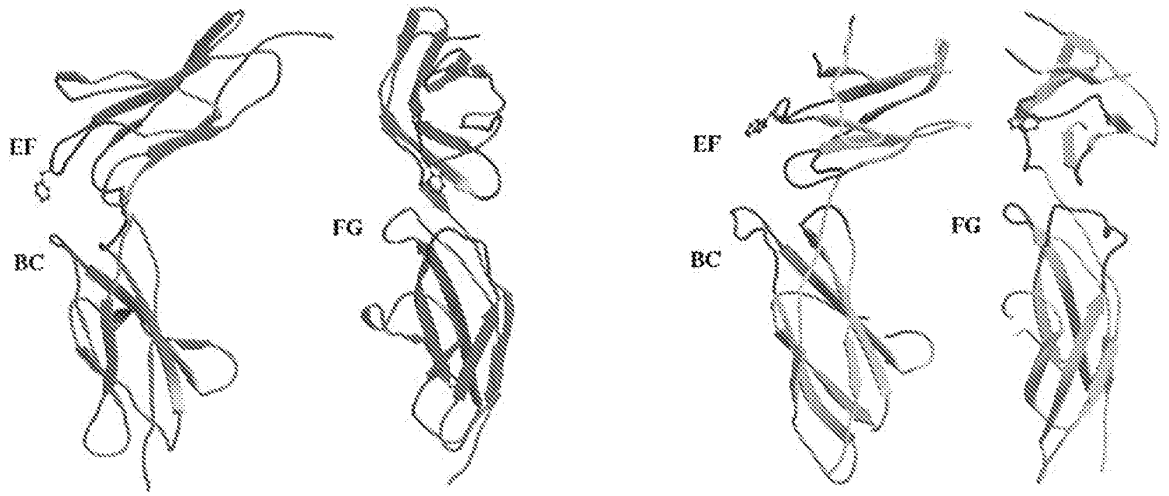

Fig. 5. The putative ligand-binding region in gp130-CHR compared with hGHR. Two orthogonal views are shown for gp130-CHR (green) and hGHR (yellow). Loops implicated in ligand binding for the hGH–hGHR complex, and their equivalents in the gp130-CHR structure, are denoted in red. The side chains of the structurally equivalent residues Phe169 and Trp104 are shown in ball and stick representation.

complex (de Vos *et al.*, 1992) shows that D2 of each hGHR partner receptor is in apposition, forming a receptor dimer interface. It is likely, therefore, that non-conservative mutations in the dimer interface could disrupt the formation of a high affinity complex. It may therefore be anticipated that the analogous dimer interface region of gp130 (formed from the AB loop and strand E of D2) may also include sites of receptor–receptor recognition. It is of interest, therefore, that amongst the few species-conserved residues exhibiting significant access to solvent, five (Glu213, Leu214, Lys219, Gln265 and Asp266) are located in this region (Figure 4), suggesting that they may be involved in the formation of the receptor dimer interface.

Conclusions

The gp130-CHR structure reported here is the first for an unliganded receptor in this superfamily. As discussed previously, the observed, L-shaped, quaternary structure and the nature of the interdomain linker region imply that there is little domain reorientation on ligand binding. Gp130 is competent to bind several different ligands; the current structure implies that it does so with essentially identical global structure. Conformational variability in the ligand-binding loops could be envisaged as one potential mechanism for such adaptability in ligand recognition. Indeed, the BC loop of D2 in hGHR adopts a different conformation on binding site I or site II of hGH (de Vos *et al.*, 1992). The unliganded structure of gp130, however, provides little indication of any potential for such a mechanism, with the D2 BC loop showing a single rigid conformation. Clearly the structure of unliganded gp130-CHR represents only the first stage in studies of these recognition events. A comparison of the conformations of various interdomain linkers with that of the N-terminal portion of the polypeptide in gp130-CHR does, however, provide some pointers to the structure/function of cytokine receptors containing an additional Ig-like domain. The gp130 Ig-D1 linker bears closest similarity in relative position and overall conformation to that of the linker between variable and constant domains in the light chain of Fab NEW (Saul and Poljak, 1992, comparison not shown). The implied positioning of the gp130 Ig-like domain is fully consistent with previously proposed models for this domain's role in multimeric recognition complexes (e.g. Simpson *et al.*, 1997), but a detailed analysis must await further structural studies.

Materials and methods

Native and selenomethionyl protein production

Full details of the expression, purification and functional characterization of gp130-CHR are reported elsewhere (D.Staunton, K.R.Hudson and J.K.Heath, in preparation). Briefly, the 25 kDa CHR of human gp130 (residues 100–303) was expressed as a folded fusion protein secreted into the periplasmic space of *E.coli* by the pMALp2 expression vector (New England Biolabs). Purification of the MBP–gp130-CHR protein fusion was achieved by ion exchange chromatography. The fusion protein was cut with rhinovirus 3c protease and the gp130-CHR separated from the MBP by high resolution ion exchange chromatography. The activity of the purified gp130-CHR was determined by its ability to bind the cytokine OSM as followed by surface plasmon resonance analysis (BIAcore).

The selenomethionyl form (SeMet-gp130-CHR) was produced by similar methods but in the presence of selenomethionine-enriched media. As for the native protein, the gp130-CHR fragment fused with MBP was expressed in *E.coli* strain HW1110 using the pMALp2 vector. The bacteria were grown in defined medium prepared as described by LeMaster and Richards (1985). A 50 ml pre-culture grown in LeMaster's medium supplemented with 1 mg/ml thiamine and 50 μg/ml carbenicillin was used to innoculate 4 l of media additionally supplemented with 50 mg/l seleno-L-methionine (Sigma). The bacteria grew at 37°C with a doubling time of ~150 min. At an absorbance of 1.5/cm (at 600 nm), the culture was induced with 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and the incubation temperature reduced to 25°C. The cultures were harvested after a further 3 h of growth when the absorbance had reached 3.0/cm. The purification was performed as for the native protein except that an additional step of amylose resin (New England Biolabs) affinity chromatography was introduced after the initial ion exchange to remove contaminating proteins from the fusion protein and all buffers contained 1 mM dithiothreitol and 1 mM EDTA to prevent oxidation of the selenomethionine.

Mass determinations by electrospray ionization mass spectrometry were performed on a VG BioQ mass spectrometer. Analysis of the gp130-CHR was consistent with a polypeptide of the expected sequence (calculated mass 25 210 Da, observed mass 25 209 ± 5 Da). Gp130-CHR contains two methionine residues, and a comparison of the

measured mass of the selenoderivative indicated that the SeMet-gp130-CHR had 95% seleno-L-methionine incorporation in place of normal methionine. Forms corresponding to mono- and non-substituted gp130-CHR were not detected in the purified sample. The SeMet-gp130-CHR had an affinity for OSM identical to that of the native gp130-CHR, with a dissociation constant of 50 nM as followed by surface plasmon resonance analysis (BIAcore).

Crystallization

Both wild-type and SeMet-gp130-CHR were crystallized at 4°C using the hanging drop vapour diffusion method over a reservoir solution containing 1.8–2.1 M ammonium sulfate, 0.1 M Tris pH 8.0. Typically, 1.5 µl of the protein solution (12 mg/ml in 20 mM Tris pH 8.0) was mixed with an equal volume of the reservoir solution. SeMet-gp130-CHR crystals were slightly smaller than wild-type ($0.7\times0.4\times0.2$ mm^3) but, when exposed to synchrotron radiation, showed ordered Bragg diffraction to about the same resolution (2 Å). The crystals are of the $C222_1$ space group with unit cell dimensions a = 84.48 Å, b = 132.29 Å and c = 121.93 Å. There are two molecules per asymmetric unit and the crystal solvent content is ~62%.

Data collection

All data sets were collected using synchrotron radiation. Crystals used for cryo-crystallographic data collection were transfered to mother liquor containing 25% glycerol, flash-cooled in liquid propane and stored at –170°C in liquid nitrogen. Data for MAD-based phase determination were collected from one cryo-cooled SeMet-gp130-CHR crystal at the BM14 beam line of the ESRF. The characteristics of the Se absorption edge for this crystal were determined by a fluorescence scan, and five wavelengths were selected for data collection at points corresponding to the peak (maximum f′, λ = 0.9790 Å), inflection point (minimum f′, λ = 0.9791 Å), and two remote energies, below at λ = 0.9793 Å and above the absorption edge at λ = 0.9535 Å. A fifth data set was collected just above the maximum f′ at λ = 0.9789 Å. Diffraction data sets were collected, at each wavelength in turn, over the same 95° φ range as 0.5° oscillation images using a CCD detector (Hammersley *et al.*, 1994; Moy, 1994). A single wavelength (λ = 1.03 Å) high resolution (2 Å) data set subsequently was collected as 1° oscillation images from a cryo-cooled wild-type gp130-CHR crystal at the same beam line using an imaging plate detector (MarResearch 30 cm diameter).

The diffraction data were autoindexed and integrated using program DENZO. Data at each wavelength were scaled separately and merged (preserving Bjivoet pairs for the MAD data) using SCALEPACK (Otwinowski and Minor, 1997). Crystallographic data collection statistics are reported in Table I.

Structure determination

The Se positions were determined from the SeMet-gp130-CHR data sets by manual inspection of difference and anomalous Patterson syntheses and confirmed using the program SHELX (Sheldrick *et al.*, 1993). The four Se sites were refined in program MLPHARE, and MAD phases were calculated to 2.9 Å resolution (figure of merit 0.48). An initial map based on these phases and F_{obs} from the λ = 0.9791 Å data set showed clear electron density for both copies of gp130-CHR in the asymmetric unit. Density modification using solvent flattening and histogram matching procedures as implemented in the program DM (Cowtan, 1994; CCP4 suite of programs) yielded a high quality electron density map which was used in the interactive graphics program O (Jones *et al.*, 1991) to trace the gp130-CHR structure (Figures 1 and 3B).

Refinement and structural analysis

Refinement was carried out using standard protocols in the program X-PLOR (Brünger, 1992). A model for copy 1 of the two molecules in the asymmetric unit was built using program O and a model for copy 2 generated from these coordinates by application of non-crystallographic symmetry operators. The model was refined initially at 2.5 Å resolution against the SeMet-gp130-CHR λ = 0.9535 data set. Tight non-crystallographic symmetry restraints were maintained between the two molecules, and 7.5% of the data were reserved for R_{free} monitoring. Once available, the 2.0 Å resolution native data set was used for all subsequent refinement with an extension of the test array for the cross-validation. Inclusion of a bulk solvent correction allowed all measured data for 30.0–2.0 Å resolution to be used. In the final stages of refinement, non-crystallographic symmetry restraints were released and ordered water molecules were modelled.

For one of the copies in the asymmetric unit, significant electron density is present for all 204 residues of the human gp130-CHR plus all three N-terminal residues and eight of the C-terminal residues derived from the expression construct. The second copy is less well ordered, lacking clear electron density for the N-terminal residues up to and including gp130 residue Ser100 and gp130 residues 212–213 in a loop region of the second domain; seven residues from the expression construct are visible at the C-terminus of this molecule. For this final model, no non-glycine residues fall in the disallowed regions of the Ramachandran plot. Refinement and model statistics are reported in Table II.

Table II. gp130-CHR structural refinement statistics

Resolution range (Å)	30–2.0
Completeness (%)	95.0
No. of reflections ($F>0$)	44 627
R_{cryst} (%)	21.5
R_{free} (%)	25.0
No. of non-hydrogen atoms	
Protein	3359
Water	287
Sulfate	30
R.m.s.d. from ideality	
Bond lengths (Å)	0.006
Bond angles (°)	1.40
Dihedrals (°)	25.36
Improper (°)	1.11
Average B-factor (Å^2)	
Main chain	19.5
Side chain	20.3
Water	19.1

$R_{cryst} = \Sigma||F_{obs}| - |F_{calc}||/\Sigma|F_{obs}|$.
R_{free} is as for R_{cryst} but calculated for a test set comprising reflections not used in the refinement (7.5%).

Atomic coordinates

Atomic coordinates for gp130-CHR have been deposited with the Protein Data Bank, Brookhaven National Laboratory, USA.

Acknowledgements

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IL-11 Receptor α in the Pathogenesis of IL-13-Induced Inflammation and Remodeling[1]

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IL-13 is a major stimulator of inflammation and tissue remodeling at sites of Th2 inflammation. In Th2-dominant inflammatory disorders such as asthma, IL-11 is simultaneously induced. However, the relationship(s) between IL-11 and IL-13 in these responses has not been defined, and the role(s) of IL-11 in the genesis of the tissue effects of IL-13 has not been evaluated. We hypothesized that IL-11, signaling via the IL-11Rα-gp130 receptor complex, plays a key role in IL-13-induced tissue responses. To test this hypothesis we compared the expression of IL-11, IL-11Rα, and gp130 in lungs from wild-type mice and transgenic mice in which IL-13 was overexpressed in a lung-specific fashion. We simultaneously characterized the effects of a null mutation of IL-11Rα on the tissue effects of transgenic IL-13. These studies demonstrate that IL-13 is a potent stimulator of IL-11 and IL-11Rα. They also demonstrate that IL-13 is a potent stimulator of inflammation, fibrosis, hyaluronic acid accumulation, myofibroblast accumulation, alveolar remodeling, mucus metaplasia, and respiratory failure and death in mice with wild-type IL-11Rα loci and that these alterations are ameliorated in the absence of IL-11Rα. Lastly, they provide insight into the mechanisms of these processes by demonstrating that IL-13 stimulates CC chemokines, matrix metalloproteinases, mucin genes, and gob-5 and stimulates and activates TGF-β1 via IL-11Rα-dependent pathways. When viewed in combination, these studies demonstrate that IL-11Rα plays a key role in the pathogenesis of IL-13-induced inflammation and remodeling. ***The Journal of Immunology,*** **2005, 174: 2305–2313.**

Interleukin-13 is a pleiotropic 12-kDa product of a gene on chromosome 5 at q31 that is produced in large quantities by stimulated Th2 cells. It was originally described as an IL-4-like molecule based on shared effector properties, including the ability to stimulate IgE production. Subsequent studies demonstrated that IL-13 and IL-4 often play distinct roles in biology. A prominent aspect of this distinction is the appreciation that IL-4 plays a key role in Th2 cell differentiation and response generation, whereas IL-13 contributes as the major effector of Th2 inflammation and tissue remodeling (1–4). In accord with these observations, IL-13 dysregulation has been documented, and IL-13 has been implicated in the pathogenesis of a variety of diseases characterized by inflammation and tissue remodeling, including asthma, idiopathic pulmonary fibrosis, scleroderma, viral pneumonia, hepatic fibrosis, nodular sclerosing Hodgkin's disease, and chronic obstructive pulmonary disease (COPD)[4] (1–11). Studies from our laboratory and others have demonstrated that IL-13 mediates its tissue effects by activating a broad array of downstream target genes, including chemokines, matrix metalloproteinases (MMPs), TGF-β1, and chitinases (12–16). The importance of IL-6-type cytokines in the generation of the effects of IL-13, however, have not been investigated.

IL-11 is a multifunctional IL-6-type cytokine with diverse biologic properties, including the ability to stimulate hemopoiesis, thrombopoiesis, megakaryocytopoiesis, and bone resorption; regulate macrophage differentiation; and confer mucosal protection after chemotherapy and radiation therapy (17–22). These effects are mediated by a multimeric receptor that contains a ligand-binding α subunit, IL-11Rα, and the ubiquitous β subunit, gp130, that triggers intracellular signaling (18, 23, 24). Previous studies from our laboratory and others demonstrated that, like IL-13, IL-11 is expressed in an exaggerated fashion in the dysregulated Th2 response in the asthmatic airway (25). Although IL-11 can inhibit Th1 responses, inhibit the production of Th1-related cytokines such as IL-12, and shift inflammation in a Th2 direction (22, 26–29), little else is known about the role(s) of IL-11 in the generation and/or expression of Th2 tissue responses. In particular, interactions between IL-11 and IL-13 have not been defined, and a role for IL-11 in the genesis of IL-13-induced pathologies has not been established.

We hypothesized that IL-11 signaling plays a key role in IL-13-induced Th2 inflammation. To test this hypothesis, we characterized the expression of IL-11, IL-11Rα, and gp130 in lungs from wild-type (WT) mice and mice in which IL-13 was overexpressed in a lung-specific fashion. We also characterized the effects of a null mutation of IL-11Rα on the tissue effects of transgenic IL-13. These studies demonstrate that IL-13 is a potent stimulator of IL-11 and IL-11Rα. They also demonstrate that IL-11Rα plays a key role in IL-13-induced inflammation, fibrosis, hyaluronic acid

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[4] Abbreviations used in this paper: COPD, chronic obstructive pulmonary disease; BAL, bronchoalveolar lavage; HA, hyaluronic acid; MMP, matrix metalloproteinase; Tg, transgenic; Timp, tissue inhibitor of MMP; WT, wild type; TARC, thymus and activation-regulated chemokine.

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(HA) accumulation, myofibroblast accumulation, alveolar remodeling, mucus metaplasia, and respiratory failure and death. Lastly, they provide insights into the mechanisms of these processes by demonstrating that IL-13 stimulates CC chemokines, MMPs, mucin genes, and gob-5 and stimulates and activates TGF-β1 via IL-11Rα-dependent pathways.

Materials and Methods

Overexpression transgenic mice

CC10-IL-13 transgenic mice were generated in our laboratory, bred onto a C57BL/6 background, and used in these studies. These mice use the Clara cell 10-kDa protein (CC10) promoter to target IL-13 to the lung. The methods used to generate and characterize these mice were described previously (30). In this modeling system, IL-13 caused a mononuclear cell- and eosinophil-rich tissue inflammatory response, alveolar enlargement, subepithelial and parenchymal fibrosis, mucus metaplasia, and respiratory failure and death, as previously described (12, 13, 30).

IL-11Rα-null mice (IL-11R$\alpha^{-/-}$) were provided by Drs. L. Robb and C. Glenn Begley (Walter and Eliza Hall Institute, Victoria, Australia) (31, 32). These mice were bred for more than eight generations onto a C57BL/6 genetic background. CC10-IL-13 mice with WT$^{+/+}$ and null$^{-/-}$ IL-11Rα loci were generated by breeding the IL-13 transgenic (Tg^{+}) mice with the IL-11R$\alpha^{-/-}$ animals. Genotyping was accomplished as previously described (30, 32). Littermate control WT mice with (+/+) or without (−/−) IL-11Rα loci were used as controls.

Bronchoalveolar lavage (BAL)

Lung inflammation was assessed by BAL as previously described (13, 33). The BAL samples from each animal were then pooled and centrifuged. The number and types of cells in the cell pellet were determined as previously described (12, 13). The supernatants were stored at −20°C until used.

Lung volume and morphometric assessments

Animals were anesthetized, the trachea was cannulated, and the lungs were removed and inflated with PBS at 25 cm. The size of each lung was evaluated via volume displacement, and alveolar size was estimated from the mean chord length of the airspace, as previously described by our laboratory (13). Chord length increases with alveolar enlargement.

Histologic evaluation

Animals were killed, a median sternotomy was performed, and right heart perfusion was accomplished with calcium- and magnesium-free PBS. The heart and lungs were then removed en bloc, inflated at 25 cm pressure with neutral-buffered 10% formalin, fixed in 10% formalin, embedded in paraffin, sectioned, and stained. H&E, Mallory's Trichrome, and periodic acid-Schiff with diastase stains were performed at Yale University School of Medicine.

mRNA analysis

The levels of mRNA encoding IL-11 and IL-11Rα were evaluated with a commercial RNase protection assay (BD RiboQuant; BD Biosciences) as described by the manufacturer. Other mRNA levels were evaluated by RT-PCR analysis as previously described (13). The primers used have been described previously (12, 13, 15, 16). For each cytokine, the optimal numbers of cycles that will produce a quantity of cytokine product that is directly proportional to the quantity of input mRNA was determined experimentally. β-Actin was used as an internal standard. Amplified PCR products were detected using ethidium bromide gel electrophoresis, quantitated electronically, and confirmed by nucleotide sequencing.

Quantification of IL-13 and chemokines

BAL IL-13 and chemokine levels were quantitated using commercial ELISA kits (R&D Systems) according to the manufacturer's instructions.

Immunohistochemistry

α-Smooth muscle actin and myosin H chain staining cells were evaluated by immunohistochemistry as previously described by our laboratory (15). The primary Abs were obtained from DakoCytomation. Specificity was assessed by comparing the staining of serial sections that were incubated in the presence and the absence of the primary Ab.

Quantification of lung collagen

Collagen content was determined biochemically by quantifying total soluble collagen using the Sircol collagen assay kit (Biocolor) according to the manufacturer's instructions (15). The data are expressed as the collagen content of the entire right lung. Collagen was also assessed morphometrically using picosirius red staining, performed as described previously by our laboratory (15). These data are expressed as the percentage of the histologic section with picosirius red staining.

Quantification of HA

The levels of BAL HA were measured using a competitive ELISA using biotinylated HA-binding protein as described previously (34, 35). Microtiter plates were coated with HA by combining rooster comb HA, carbodiimide HCl, and HCl. Samples were incubated with biotinylated HA-binding protein for 1 h and then added to the wells. The plate was then agitated, washed, developed with HRP-streptavidin, and exposed to peroxidase substrate for 30 min. OD at 405 nm was evaluated. Samples were compared with a simultaneously performed standard curve.

TGF-β bioassay

The levels of total and bioactive TGF-β1 were evaluated by ELISA (R&D Systems) using untreated and acid-treated BAL fluids according to the manufacturer's instructions.

Murine 100% O_2 exposure

Adult 6- to 8-wk-old Tg^{-} and Tg^{+} mice with WT or null mutant IL-11Rα loci were exposed to room air (controls) or continuously to 100% O_2 in a Plexiglas chamber as previously described (19, 36). All protocols were reviewed and approved by the institutional animal care and use committee at Yale University School of Medicine.

Statistics

Normally distributed data are expressed as the mean ± SEM and assessed for significance by Student's t test or ANOVA as appropriate. Data that were not normally distributed were assessed for significance using the Wilcoxon rank-sum test.

Results

Effect of IL-13 on IL-11 and IL-11R expression

Studies were undertaken to define the effects of IL-13 on IL-11 and its receptor components in murine lung. These studies demonstrated that transgenic IL-13 is a potent stimulator of the expression of IL-11 and IL-11Rα. These effects were readily apparent at all time points evaluated (1–4 mo; Fig. 1 and data not shown). The induction of IL-11 was associated with similar increases in the levels of mRNA encoding other IL-6-type cytokines, including IL-6 and LIF (Fig. 1). A modest increase in gp130 expression was also observed (Fig. 1). Similar alterations in M-CSF, GM-CSF, stem cell factor, L32, and GAPDH, however, were not found. The alterations in IL-11Rα were also at least partially specific, because

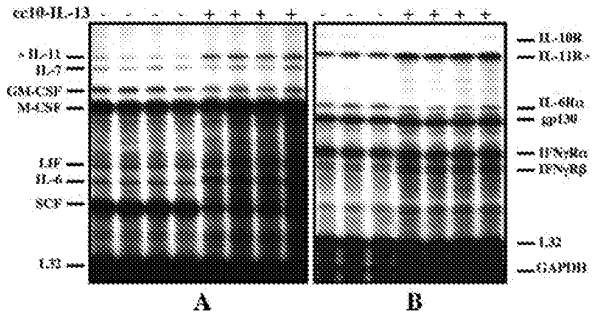

FIGURE 1. IL-13 regulation of IL-11Rα and IL-11. Lungs were obtained from 2-mo-old CC10-IL-13 Tg^{-} and Tg^{+} mice, and the levels of mRNA encoding the noted cytokines, proteins, and receptors were evaluated using RNase protection. Each lane represents an individual animal.

comparable alterations in the expression of IL-6Rα and IFN-γRα were not observed (Fig. 1). These studies demonstrate that IL-13 is a potent stimulator of IL-11 and the α subunit of its receptor in murine lung.

Role of IL-11R signaling in IL-13-induced inflammation

To address the importance of IL-11 in the pathogenesis of IL-13-induced tissue inflammation, CC10-IL-13 transgenic mice were bred with IL-11Rα$^{-/-}$ mice. The inflammatory responses in IL-13 Tg^{+} mice with WT and null IL-11Rα loci were then compared. As previously reported (12, 30), IL-13 was a potent stimulator of tissue inflammation that caused a progressive increase in the accumulation of macrophages, lymphocytes, and eosinophils in the tissues and BAL fluids of IL-13 Tg^{+} mice with normal IL-11Rα loci. In the absence of IL-11Rα, an impressive decrease in this inflammatory response was noted. In 2- and 4-mo-old mice, impressive decreases in BAL total cell, macrophage, and eosinophil recovery were noted (Fig. 2, *A* and *B*). A similarly, impressive decrease in tissue inflammatory cell accumulation was apparent (Fig. 2*C* and data not shown). In BAL and tissues, compensatory increases in neutrophils were not noted (Fig. 2).

Role of IL-11Rα in IL-13-induced chemokine elaboration

Previous studies from our laboratory demonstrated that IL-13 induces its tissue alterations in part via the induction of a wide array of CC chemokines (12). To investigate the mechanism by which IL-11Rα deficiency diminished IL-13-induced inflammation, we compared the expression of selected chemokines in IL-13 Tg^{+} mice with WT and null IL-11Rα loci. In Tg^{-} mice with WT or null IL-11Rα loci, the levels of mRNA encoding MCP-1/CCL2, MCP-2/CCL8, MCP-3/CCL7, MIP-1α/CCL-3, MIP-1β/CCL4, MIP-2/CXCL-2/3, MIP-3α/CCL20, C10/CCL-6, eotaxin/CCL-11, eotaxin-2/CCL21, and thymus and activation-regulated chemokine (TARC)/CCL17 were comparable and in many cases were near or below the limits of detection of our assays (Fig. 3*A*). As previously reported (12, 37), IL-13 increased the levels of mRNA encoding these chemokine moieties in Tg^{+} mice with WT IL-11Rα loci (Fig. 3*A*). In contrast, in the absence of IL-11Rα, the ability of IL-13 to induce MCP-1/CCL2, MCP-2/CCL8, MCP-3/CCL7, MIP-1α/CCL3, MIP-1β/CCL4, MIP-2/CXCL2–3, MIP-3α/CCL20, C10/CCL6, eotaxin/CCL11, eotaxin-2/CCL21, and TARC/CCL17 was markedly diminished (Fig. 3*A*). In accord with these mRNA alterations, comparable alterations in BAL MCP-1/CCL2, MIP-1α/CCL-3, and eotaxin/CCL-11 protein were observed (Fig. 3, *B–D*). Thus, IL-11Rα plays an essential role in the stimulation of selected chemokines by IL-13.

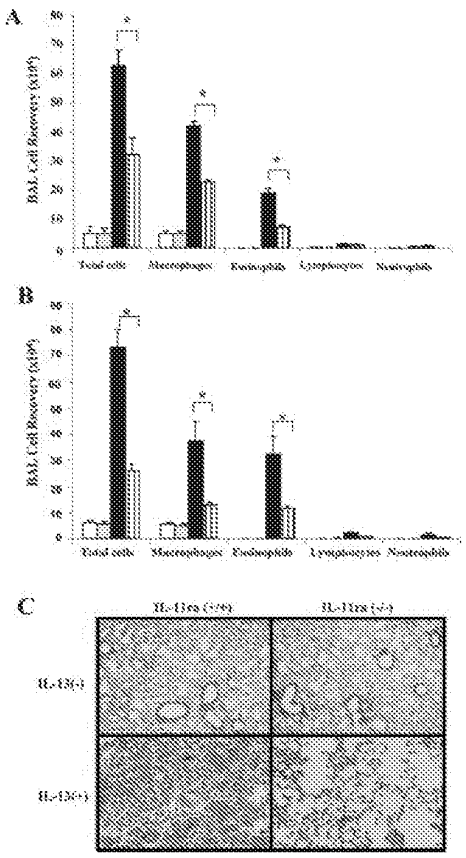

FIGURE 2. Role of IL-11Rα in IL-13-induced inflammation. *A* and *B*, The BAL cell recoveries of Tg^{-}/IL-11Rα$^{+/+}$ mice (□), Tg^{-}/IL-11Rα$^{-/-}$ mice (▨), Tg^{+}/IL-11Rα $^{+/+}$ mice (■), and Tg^{+}/IL-11Rα $^{-/-}$ mice (▩) at 2 (*A*) and 4 (*B*) mo of age are compared. The histologic effects in 4-mo-old mice are illustrated in *C* (original magnification, ×10). *, $p < 0.01$.

Role of IL-11Rα in IL-13-induced fibrosis and HA and myofibroblast accumulation

Quantitative morphometric, biochemical, and immunohistochemical approaches were used to define the role of IL-11Rα in IL-13-induced pulmonary fibrosis and HA and myofibroblast accumulation. In these studies, we compared these collagen, HA, and cellular parameters in IL-13 Tg^{+} mice with WT and null IL-11Rα loci. Similar amounts of collagen and BAL HA and similar numbers of anti-smooth muscle actin-staining parenchymal cells were noted in lungs from WT littermate control mice and IL-11Rα$^{-/-}$ animals (Fig. 4). In WT mice, IL-13 caused an impressive increase in lung collagen content (Fig. 4, *A* and *B*) and BAL HA levels (Fig. 4*C*) that could be easily determined by histochemical and biochemical measurement techniques. In addition, IL-13 increased the accumulation of parenchymal myofibroblast-like cells that contained anti-smooth muscle actin, but did not stain with Abs against smooth muscle myosin (Fig. 4*D* and data not shown). In contrast, the levels of IL-13-induced collagen and HA were significantly reduced in lungs from Tg^{+} mice with null vs WT IL-11Rα loci (Fig. 4, *A–C*). Myofibroblast accumulation was similarly decreased in lungs from IL-13 Tg^{+}/IL-11Rα$^{-/-}$ mice compared with Tg^{+}/IL-11Rα$^{+/+}$ animals (Fig. 4*D*). Interestingly, the anti-smooth muscle actin staining of vascular smooth muscle cells was not altered in the absence of IL-11Rα (Fig. 4*D*). Thus, IL-11 signaling plays a critical role in IL-13-induced tissue fibrosis and HA and myofibroblast accumulation.

Role of IL-11Rα in IL-13-induced production and activation of TGF-β1

Previous studies from our laboratory demonstrated that the fibrotic effects of IL-13 are mediated by its ability to induce and activate TGF-β1 and that this activation is mediated to a great extent by MMP-9 (15). To define the importance of IL-11Rα in these responses, we evaluated the TGF-β1 production of Tg^{+} mice with WT and null IL-11Rα loci. In mice with a WT IL-11Rα locus, IL-13 was a potent stimulator of the levels of mRNA encoding TGF-β1, TGF-β2, and TGF-β3 (Fig. 5*A*). IL-13 also augmented MMP-9 mRNA accumulation (Fig. 5*A*). In accord with these observations, IL-13 increased the levels of spontaneously activated and total TGF-β1 protein in BAL fluids from these animals (Fig. 5, *B* and *C*). In all cases, these inductive effects appeared to be

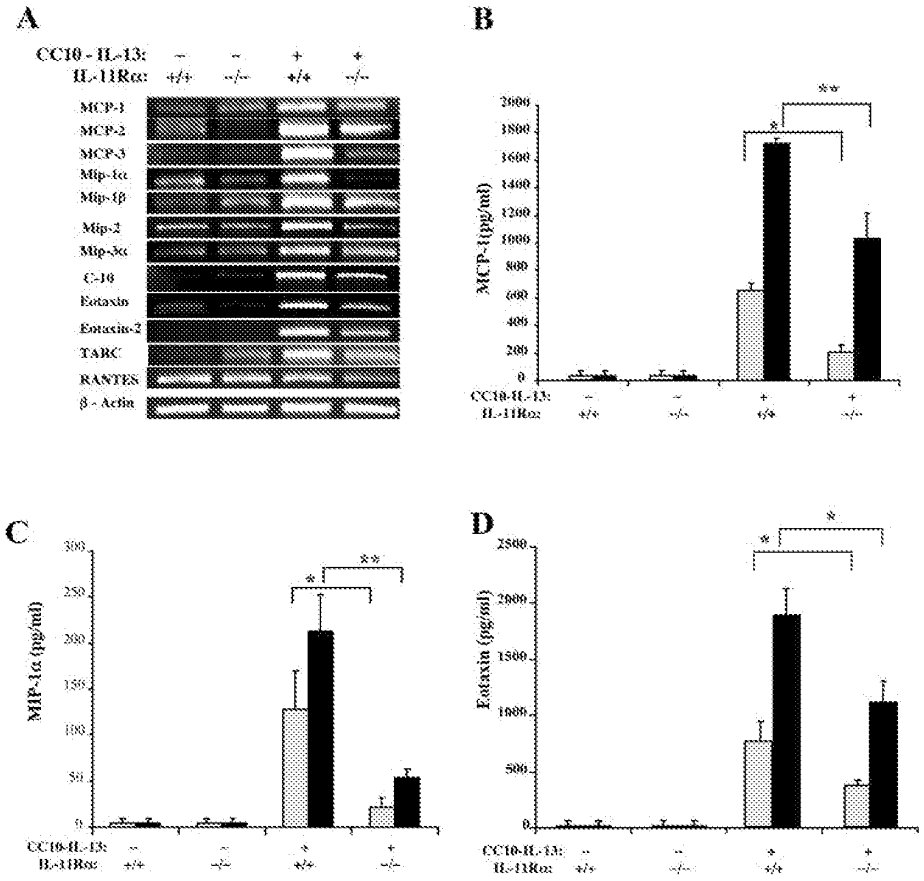

FIGURE 3. Role of IL-11Rα in IL-13-induced chemokine stimulation. *A*, Comparison of the levels of mRNA encoding the noted chemokines in lungs from Tg^- and Tg^+ mice with +/+ and −/− IL-11Rα loci. *B–D*, Levels of MCP-1, MIP-1α, and eotaxin protein were assessed by ELISA in BAL fluids from 2-mo-old (▨) and 4-mo-old (■) mice. The evaluations in *A* are representative of four similar evaluations. *B–D*, Each value is the mean ± SEM of evaluations in a minimum of five mice. *, $p < 0.05$; **, $p < 0.01$.

IL-11Rα-dependent, because the levels of mRNA encoding TGF-β1, -β2, and -β3 and MMP-9 and the production and activation of TGF-β1 were significantly decreased in IL-11Rα-null mutant mice (Fig. 5). Thus, IL-13 stimulates and activates TGF-β1 and induces production of the TGF-β1 activator, MMP-9, via an IL-11Rα-dependent mechanism.

Role of IL-11Rα in IL-13-induced alveolar remodeling

To define the role(s) of IL-11Rα in the pathogenesis of IL-13-induced alveolar remodeling, we compared the alterations in lung volume and alveolar size in IL-13 Tg^+ mice with WT and null IL-11Rα loci. In accord with previous observations (13), IL-13 caused an impressive increase in these parameters in lungs from mice with WT IL-11Rα loci (Fig. 6, *A* and *B*). In contrast, these effects of IL-13 were significantly diminished in mice with null IL-11Rα loci (Fig. 6, *A* and *B*). Thus, IL-11Rα plays a key role in this remodeling response.

Effects of IL-11Rα deficiency on lung proteases

To determine whether a deficiency of IL-11Rα could modulate the IL-13-induced alveolar phenotype by decreasing the production of respiratory proteases, we compared the levels of mRNA encoding lung-relevant MMPs and cathepsins in WT and IL-11Rα$^{-/-}$ mice. As noted above (Fig. 5*A*), IL-13 is a potent stimulator of MMP-9, and this inductive event was mediated via an IL-11Rα-dependent pathway. As shown in Fig. 6*C*, IL-13 was also a potent stimulator of MMP-2, MMP-12, tissue inhibitor of MMP (Timp)-1, Timp-2, Timp-3, Timp-4, cathepsin K, cathepsin S, cathepsin B, and cathepsin L. Interestingly, the induction of MMP-2, MMP-12, Timp-1 to -4, cathepsin K, and cathepsin B was decreased in the absence of IL-11Rα (Fig. 6*C*). Thus, in the setting of a deficiency of IL-11Rα, IL-13 is unable to optimally stimulate lung proteases.

Role of IL-11Rα in IL-13-induced mucus metaplasia

Studies were next undertaken to determine whether IL-11Rα played an important role in the pathogenesis of IL-13-induced mucus metaplasia. In these studies we compared mucin gene expression in Tg^+ mice with WT and null IL-11Rα loci. The expression of gob-5, a calcium-activated chloride channel involved in the mucus response (38), was also evaluated. In lungs from Tg^- mice with WT or null IL-11Rα loci, the levels of expression of Muc-5ac and gob-5 were at or near the limits of detection in our assay (Fig. 7). In contrast, IL-13 was a potent stimulator of muc-5AC and gob-5 in murine lung (Fig. 7). Interestingly, the stimulation of muc5AC and gob-5 gene expression were diminished in Tg^+ mice with null mutant IL-11Rα loci (Fig. 7). These studies demonstrate that IL-11 plays an important role in the pathogenesis of IL-13 stimulation of mucin and gob-5 gene expression.

Role of IL-11Rα in IL-13-induced respiratory death

In CC10-IL-13 Tg^+ mice, progressive lung pathology is noted. As a result, these mice die prematurely from a fibrodestructive, inflammatory alveolar filling process that abrogates normal respiratory function (12). To define the role of IL-11Rα in this fatal response, we compared the survival of IL-13 Tg^+ mice with WT and null IL-11Rα loci. Tg^+ mice with IL-11Rα$^{+/+}$ loci started to die at ~100 days of age, and 100% of these animals were dead by 4.1 mo of age (Fig. 8). As shown in Fig. 8, a deficiency of IL-11Rα

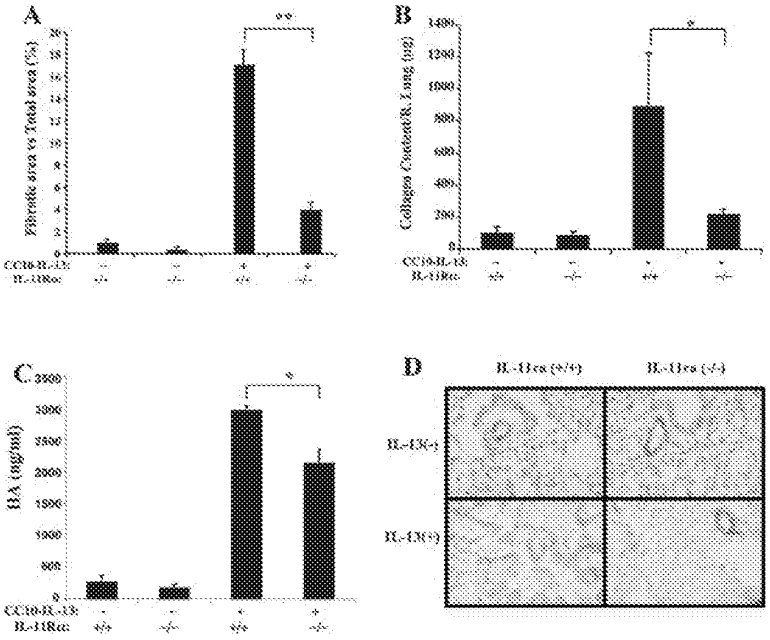

FIGURE 4. Role of IL-11Rα in IL-13-induced fibrosis and HA and myofibroblast accumulation. The collagen content of lungs from 4-mo-old IL-13 Tg^- and Tg^+ mice with +/+ and −/− IL-11Rα loci were compared using Picosirius Red (*A*) and Sirchol (*B*) collagen evaluations. *C*, HA content of BAL fluids from Tg^- and Tg^+ mice with WT and null IL-11Rα loci. *D*, Comparison of α-smooth muscle actin staining of lungs from 4-mo-old IL-13 Tg^+ mice with +/+ and −/− IL-11Rα loci. *A–C*, Each value represents the mean ± SEM of evaluations in a minimum of five mice. *D*, Representative of four similar evaluations. *, $p < 0.05$; **, $p < 0.01$. N.D., none detected.

significantly improved the survival of these animals, with Tg^+/IL-11Rα$^{-/-}$ animals beginning to die at ~5.5 mo of age and many animals living to 7.8 mo of age (Fig. 8). Thus, IL-11Rα plays a critical role in the pathogenesis of the IL-13-induced pathologies that lead to the death of these animals.

Role of IL-11Rα in IL-13-induced protection in 100% O_2

Previous studies from our laboratory demonstrated that IL-13 confers an impressive level of cytoprotection in the context of hyperoxia-induced acute lung injury (19). To define the role of IL-11Rα in this protective response, we compared the survival of Tg^+ and Tg^- mice with WT and null IL-11Rα loci in 100% O_2. WT mice died after 4–6 days of exposure to 100% O_2 (Fig. 9). Interestingly, IL-11Ra$^{-/-}$ mice were more susceptible to 100% O_2 than their WT littermate controls, dying after 2–3 days of exposure to 100% O_2. As described previously (36), IL-13 Tg^+ mice with WT IL-11Rα loci lived for an extended interval, with many of these animals living for 8–12 days in hyperoxia (Fig. 9). Interestingly, a deficiency of IL-11Rα did not significantly alter this protective response (Fig. 9). Together, these studies demonstrate that IL-11Rα plays an important role in regulating the response of otherwise normal mice to hyperoxia. They also demonstrate that IL-11Rα does not play a significant role in the cytoprotection that is conferred by IL-13.

Effect of IL-11Rα deficiency on IL-13 production and receptor expression

A deficiency of IL-11Rα could modify the IL-13-induced phenotype by altering IL-13 production or the expression of the subunits that make up the IL-13R. To address the former, we compared the levels of IL-13 in BAL from Tg^+ mice with WT and null IL-11Rα loci. As shown in Fig. 10*A*, a deficiency of IL-11Rα did not alter the levels of transgenic IL-13 protein. To address the receptor issue, we compared the levels of expression of IL-4Rα and IL-13Rα1, which make up the signaling IL-13R complex, and the decoy receptor IL-13Rα2 in mice with WT and null IL-11Rα loci. The levels of mRNA encoding IL-4Rα, IL-13Rα1, and IL-13 Rα2 in Tg^- mice with WT and null IL-11Rα loci were comparable and were at or below the limits of detection of our assays (Fig. 10*B*). As previously reported (39), IL-13 was a potent stimulator of each of these moieties (Fig. 10*B*). In these experiments a deficiency of IL-11Rα caused only modest alterations in the levels of expression of IL-4Rα and IL-13Rα1 (Fig. 10*B*). Importantly, in the absence of IL-11Rα, the levels of expression of IL-13Rα2 were not augmented (Fig. 10*B*). In fact, modest decreases in the levels of expression of this decoy receptor were noted. These studies demonstrate that the amelioration of the IL-13 phenotype that is seen in IL-11Rα-null mice is not due to a decrease in IL-13 production, a decrease in IL-13Rα1-IL-4Rα receptor expression, or an increase in expression of the IL-13Rα2 decoy receptor.

Discussion

Because IL-13 and IL-11 are juxtaposed in inflammatory tissues, studies were undertaken to define the relationship(s) between these important regulatory moieties. These studies demonstrate that IL-13 is potent stimulator of IL-11 and IL-11Rα. They also demonstrate that IL-13-induced inflammation, HA accumulation, myofibroblast accumulation, tissue fibrosis, alveolar remodeling, mucin gene expression, and respiratory failure and death were all diminished in IL-13 Tg mice with null mutations of IL-11Rα. Lastly they demonstrate that IL-13 is unable to optimally stimulate inflammatory chemokines, proteases, and mucin genes and is unable to fully stimulate and activate TGF-β1 in the absence of IL-11Rα. These studies define a previously unappreciated mechanism of regulation of IL-11 and IL-11Rα. Because IL-11 is the only known ligand for the IL-11Rα-gp130 receptor complex, these studies also define a previously unappreciated role for IL-11Rα, and presumably for IL-11, in the pathogenesis of IL-13-induced tissue responses.

IL-11 was discovered as an IL-6-like molecule that stimulated the proliferation of IL-6-dependent plasmacytoma cells (40). Subsequent investigation has focused to a great extent on the effects of exogenously administered rIL-11 and its role as a potential therapeutic agent. These studies highlighted impressive effects of IL-11 on platelets, which is the basis for the approval of IL-11 by the U.S. Food and Drug Administration as a treatment that fosters

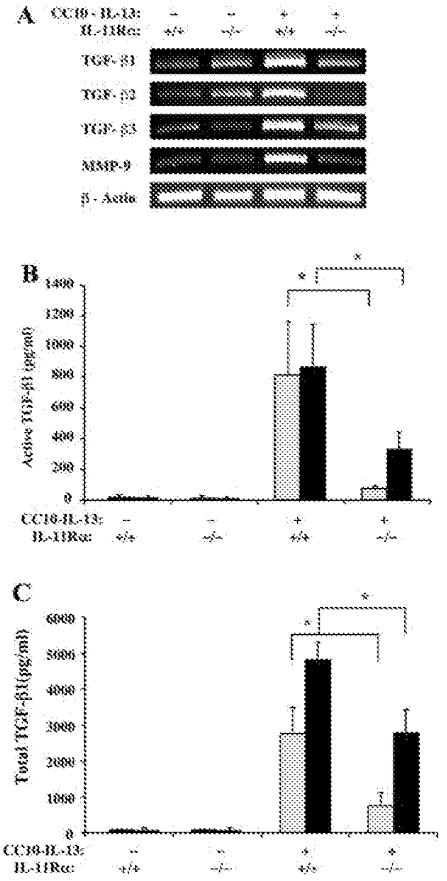

FIGURE 5. Role of IL-11Rα in IL-13 stimulation of TGF-β moieties and MMP-9. Lungs were obtained from Tg^- and Tg^+ mice with +/+ and −/− IL-11Rα loci. The levels of mRNA encoding TGF-β moieties and MMP-9 were assessed by RT-PCR (*A*), and the levels of bioactive (*B*) and total (*C*) TGF-β1 were evaluated by ELISA. *A*, Representative of four similar evaluations. *B* and *C*, Values represent the mean ± SEM of evaluation in a minimum of five mice that were either 2 (▨) or 4 (■) mo of age.

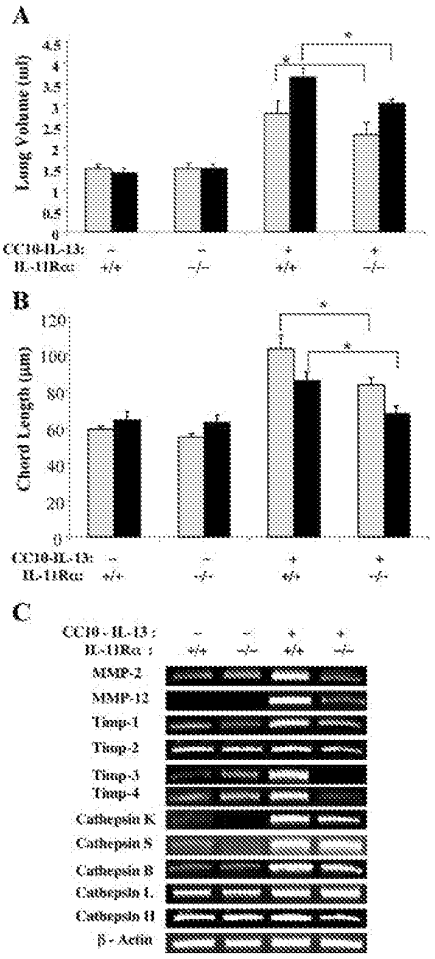

FIGURE 6. Role of IL-11Rα in IL-13-induced alveolar remodeling. Lungs were obtained from Tg^- and Tg^+ mice with +/+ and −/− IL-11Rα loci. *A* and *B*, Lungs were fixed to pressure, and lung volume (*A*) and chord length (*B*) were assessed. *C*, Levels of mRNA encoding the noted proteases and antiproteases were evaluated; representative of four similar evaluations. *A* and *B*, Values represent the mean ± SEM of evaluations in a minimum of five mice that were either 2 (▨) or 4 (■) mo of age. *, $p < 0.01$.

platelet reconstitution after bone marrow ablative therapy (17, 18). They also defined the ability of recombinant and transgenic IL-11 to confer cytoprotection and inhibit inflammation during states of mucosal/tissue injury (27, 28, 33, 41–43). These studies did not, however, address in detail the roles of endogenous IL-11 and IL-11 signaling in the generation of tissue inflammatory and extraosseous remodeling responses. The present studies provide a new level of insight into the biology of IL-11 by demonstrating that in addition to the protective effects of high concentrations of exogenous IL-11, endogenous IL-11 has important proinflammatory effects at sites of IL-13-mediated tissue inflammation. In the absence of IL-11 signaling, the ability of IL-13 to induce lymphocytic and eosinophilic tissue inflammation was markedly diminished. In accord with these findings, in the absence of IL-11Rα, IL-13 was also unable to optimally stimulate the production of the proinflammatory chemokines (MCP-1/CCL-2, MCP-2/CCL8, MCP-3/CCL7, MIP-1α/CCL3, MIP-1β/CCL4, MIP-2/CXCL2–3, MIP-3α/CCL20, C10/CCL6, eotaxin/CCL11, eotaxin 2, and TARC/CCL17) that are known to play essential roles in the generation of IL-13-induced responses (12, 37). These are the first studies to demonstrate an important proinflammatory role for IL-11/IL-11Rα in Th2 inflammation and the first to demonstrate an important role for IL-11/IL-11Rα in the induction of Th2-focused chemokines.

When combined with prior studies that demonstrate that exogenous IL-11 can prevent inflammation, the findings indicate that IL-11 has complex, context-specific effects on Th2 inflammation with the ability to augment and prevent these important responses. In many ways this is analogous to TGF-β, which can have similar bidirectional modulatory effects (44–46). This is an intriguing

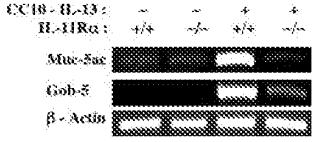

FIGURE 7. Role of IL-11Rα in IL-13 stimulation of mucin and gob-5 gene expression. Lungs were obtained from Tg^- and Tg^+ mice with +/+ and −/− IL-11Rα loci. The levels of mRNA encoding MUC-5ac and gob-5 were then evaluated. This experiment is representative of four similar evaluations.

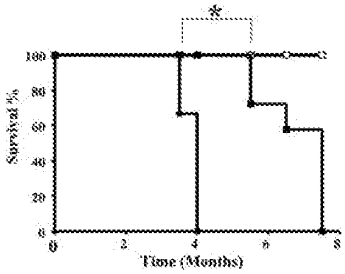

FIGURE 8. Role of IL-11Rα in IL-13-induced respiratory failure and death. The figure compares the survival of Tg^-/IL-11R$\alpha^{+/+}$ mice (○), Tg^-/IL-11R$\alpha^{-/-}$ mice (□), Tg^+/IL-11R$\alpha^{+/+}$ mice (●), and Tg^+/IL-11R$\alpha^{-/-}$ mice (■). Each value represents the survival of a minimum of eight mice. The survivals of Tg^- mice with +/+ and −/− loci are superimposed on one another. *, $p < 0.05$.

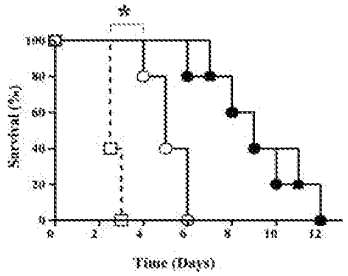

FIGURE 9. Role of IL-11Rα in IL-13-induced cytoprotection in hyperoxia. Tg^- and Tg^+ mice with +/+ and −/− IL-11Rα loci were placed in room air or in 100% O_2, and survival was assessed. The figure compares the survival of Tg^-/IL-11R$\alpha^{+/+}$ mice (○), Tg^-)/IL-11R$\alpha^{-/-}$ mice (□), Tg^+/IL-11R$\alpha^{+/+}$ mice (●), and Tg^+/IL-11R$\alpha^{-/-}$ mice (■). Each value represents the survival of a minimum of five mice. *, $p < 0.05$.

analogy, because work from our laboratory and others has demonstrated that IL-11 is potently induced by TGF-β1 in a variety of cells and tissues (18, 47–49).

Tissue fibrosis is a prominent feature of asthmatic airway remodeling and a major cause of morbidity and mortality in a variety of other pulmonary and extrapulmonary disorders. The Th2 cytokine hypothesis suggests that fibrosis is the result of Th2-dominated tissue inflammation and that IL-13 is the major mediator of these fibrotic responses (1, 6, 30, 50). We have previously demonstrated that IL-13 induces pulmonary fibrosis by inducing and activating TGF-β1 (15). We also demonstrated that this induction and activation are mediated at least in part by MCP-1/CCL2 and MMP-9, respectively (12, 15). The present studies add to our understanding of the pathogenesis of this important fibrogenic pathway by demonstrating that IL-11Rα plays a critical role in both responses. In the absence of IL-11Rα, the fibrotic effects of IL-13 were markedly diminished. In addition, the induction of MMP-9, the stimulation of MCP-1/CCL2, and the induction and activation of TGF-β1 were all markedly decreased. These observations in combination with previous reports from our laboratory demonstrating that TGF-β1 is a potent stimulator of IL-11 (18, 47–49) indicate an amplification pathway that can be activated in fibrotic tissues. During Th2 inflammation, IL-13 can stimulate IL-11 and IL-11Rα, which would, in turn, contribute to the induction of MCP-1/CCL2 and MMP-9. This would augment the production and activation of TGF-β1, which would feed back to further stimulate the production of IL-11. This amplification loop could contribute to the chronicity, progression, and/or severity of pulmonary and extrapulmonary fibrotic disorders.

Myofibroblasts are increasingly believed to play an essentially role in tissue fibrotic responses (51). Although their tissue source is still open to interpretation (52), their ability to accumulate and produce collagen and other matrix molecules at sites of injury and repair is now well accepted (51). It is also well known that myofibroblast accumulation and activation at sites of injury and repair are driven in part by TGF-β1 (45, 51). Our studies demonstrate that IL-13 induces myofibroblast accumulation and tissue fibrosis, and that IL-11Rα plays an essential role in these inductive events. These findings are in accord with previous studies from our laboratory that demonstrated that transgenic IL-11 causes pulmonary fibrosis and myofibroblast accumulation (53). Additional experimentation will be required to determine whether the inability to induce myofibroblast accumulation in the absence of IL-11Rα is the direct result of a defect in IL-11R signaling or the result of the important role that IL-11Rα plays in the induction and activation of TGF-β1.

In addition to its well-documented ability to induce eosinophilic inflammation, mucus metaplasia, and airway hyper-responsiveness (2, 3, 30), studies from our laboratory and others have also highlighted the ability of IL-13 to induce alveolar remodeling and alter protease/antiprotease balance in the lung (13). The later is the result of the ability of IL-13 to stimulate MMPs and cathepsins and inhibit α1 antitrypsin (13). Surprisingly the mechanisms of these inductive events have not been defined. The present studies address this issue by demonstrating that IL-11Rα (and presumably IL-11) play essential roles in the ability of IL-13 to cause alveolar enlargement and induce MMPs and cathepsins, because all three were decreased in IL-13 transgenic mice that were deficient in IL-11Rα. Previous studies from our laboratory also demonstrated that IL-11 has the ability to cause alveolar enlargement by blocking lung growth and development (54). When combined with the present studies, it is clear that IL-11 can alter alveolar structure via developmental and nondevelopmental pathways. The development-dependent alterations are particularly relevant to the alveolar enlargement that is seen in pediatric patients with bronchopulmonary dysplasia (55). In contrast, the development-independent effects have interesting implications with regard to the pathogenesis of pulmonary emphysema and chronic obstructive pulmonary disease. This is particularly intriguing because the subepithelial fibrosis and B cell- and T cell-rich nodules that have recently been described in tissues from patients with advanced COPD (56) are similar in many ways to subepithelial fibrotic response and B cell- and T cell-rich nodules described by our laboratory in IL-11 transgenic mice (53). In addition, polymorphisms in IL-11 have recently been shown to be associated with the development of COPD (57). These findings also have important implications for other diseases, such as periodontitis, idiopathic pulmonary fibrosis, scleroderma, and hepatic fibrosis, in which protease alterations and the exaggerated production of IL-13 or IL-11 have been noted (1, 5–11, 58).

The ability to induce goblet cell hypertrophy and mucus metaplasia is one of the most potent airway effects of IL-13 (59). Despite this potency, the mechanism of this response is poorly understood. It is clear, however, that IL-13 induces mucus metaplasia via a different mechanism than inflammation and fibrosis. This can be readily appreciated in studies that demonstrate that mucus metaplasia is not altered, whereas inflammation and fibrosis are decreased by interventions that ablate and/or neutralize specific chemokines, chemokine receptors, or TGF-β1 (12, 15, 37). An interesting feature in our studies is the demonstration that in the absence of IL-11Rα, the ability of IL-13 to stimulate mucin genes is significantly decreased. This is the first manipulation of IL-13 in

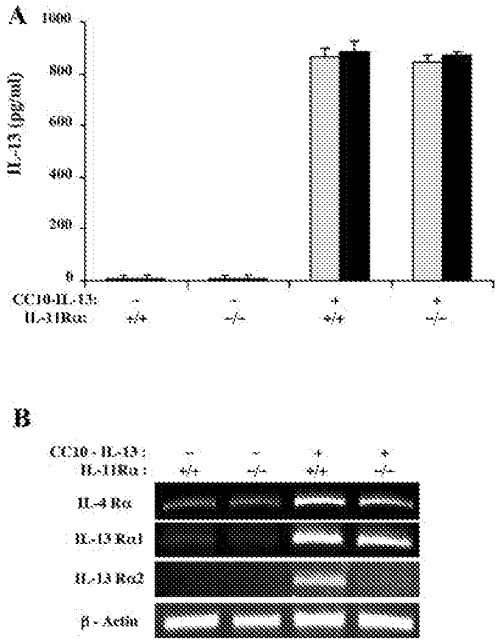

FIGURE 10. IL-11Rα regulation of BAL IL-13 and IL-13R subunit expression. BAL fluids and lungs were obtained from Tg^- and Tg^+ mice with +/+ and −/− IL-11Rα loci. The levels of BAL IL-13 (*A*) and mRNA encoding IL-13R subunits (*B*) were evaluated. *B*, Representative of four similar evaluations. The values in *A* represent the mean ± SEM of evaluations in a minimum of five mice that were 2 (▨) or 4 (■) mo of age.

vivo that simultaneously alters inflammation, fibrosis, and mucus responses. These studies demonstrate that IL-11Rα plays an important role in the pathogenesis of IL-13-induced mucus alterations. It is important to point out, however, that the role of IL-11Rα in mucus metaplasia is likely to be quite complex, because the overexpression of IL-11 by itself did not induce mucus metaplasia in the murine airway (53). Thus, IL-11Rα signaling plays an important role in IL-13 stimulation of mucin genes, but is not sufficient for the induction of airway mucus metaplasia.

In our studies the ability of IL-13 to induce inflammation, fibrosis, HA accumulation, alveolar remodeling, and respiratory failure and death was decreased in mice that were deficient in IL-11Rα. These alterations could be the result of a decrease in the production of transgenic IL-13, a decrease in IL-13 signaling, or a decrease in IL-13 effector pathway activation. The first two options are not likely, because similar levels of BAL IL-13 and similar levels of expression of the decoy receptor IL-13$R\alpha_2$ were seen in mice with wild-type and null IL-11Rα loci. Additional support comes from the specificity of the alterations that were noted, because IL-13-induced cytoprotection in hyperoxia was not altered in IL-11Rα-deficient animals. The decrease in IL-13 effector pathway activation could be caused by at least two mechanisms. First, IL-11 could be an important target of and mediator of the tissue effects of IL-13. Alternatively, IL-11 could regulate the survival of critical IL-13 target cells that are involved in the pathogenesis of IL-13-induced inflammation, remodeling, and cytokine, protease, and matrix responses. Regardless, exaggerated IL-13 production has been implicated in the pathogenesis of a variety of disorders, including asthma, COPD, pulmonary fibrosis, scleroderma, hepatic fibrosis, and nodular sclerosing Hodgkin's disease (1–11). The present studies suggest that the effector responses of IL-13 in these disorders may be beneficially controlled by interventions that block IL-11Rα and/or IL-11. This establishes the IL-11-IL-11Rα pathway as a worthwhile site for investigations designed to identify therapeutic agents that can be used to treat these and other IL-13-mediated disorders.

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REVIEW ARTICLE

Interleukin-11: Review of Molecular, Cell Biology, and Clinical Use

By Xunxiang Du and David A. Williams

FIRST ISOLATED IN 1990, interleukin-11 (IL-11) has proven to be a fascinating cytokine with pleiotropic effects on multiple tissues. Initially characterized as a hematopoietic cytokine with thrombopoietic activity, IL-11 has now been shown to be expressed and have activity in multiple other tissues, including brain, spinal cord neurons, gut, and testis. Yet to date, the physiologic role of this protein remains unknown. Our laboratory has recently generated a mutated allele of IL-11 in the mouse germline (X.D. and D.A.W., unpublished results, January 1997) and future studies of homozygous IL-11–deficient mice derived from these founder animals should illuminate the function(s) of this protein in vivo. In this article, we update current understanding of the biology of IL-11, concentrating on data published after the last comprehensive review published in 1994.[1]

CLONING AND GENOMIC CHARACTERIZATION

Human IL-11 was cloned in 1990 and details of this cloning and early work on IL-11 have been summarized previously.[1] More recently the murine IL-11 cDNA was cloned using an expression library generated from a lipopolysaccharide (LPS)-induced murine fetal thymic cell line (T2).[2] The murine IL-11 cDNA shares 80% homology with human IL-11 at the nucleotide level.[2] Both human and murine IL-11 genomic sequences consist of 5 exons and 4 introns and have been mapped to chromosome 19 at band 19q13.3-q13.4 and to the centromeric region of chromosome 7, respectively[2,3] (and M. McAndrew-Hill and D.A.W., unpublished results, June 1996). The 5′-flanking region of the human IL-11 gene contains several DNA motifs postulated to be involved in transcriptional control. A "TATA" box-like sequence, TATATAA, is located 180 nucleotides upstream from the translation initiation codon ATG.[3] A 10-bp promoter sequence (5′ GGTGAGTCAG 3′) in this region contains an activator protein-1 (AP-1) site (underlined). JunD/AP-1 complexes are responsible for the basal-level transcription of IL-11 gene in bone marrow (BM) fibroblast cells.[4] There are two polyadenylation sites located in the 3′ untranslated region (UTR) at nucleotide positions 6762 and 5591 and these alternative sites give rise to the 2.5- and 1.5-kb IL-11 mRNA transcripts expressed in several IL-1α–induced cell lines.[3,5]

PROTEIN CHARACTERIZATION

IL-11 precursor protein consists of 199 amino acids (aa), including a 21-aa leader sequence. The theoretical molecular weights of recombinant human (rh) and murine IL-11 are 19,144 daltons[6] and 19,154 daltons,[2] respectively. Mature human and primate IL-11 protein share 94% identity whereas human and murine proteins share 88% identity in the amino acid sequence.[2,6,7] Although IL-11 is rich in proline residues (12%) and lacks cysteine residues (ie, lacks potential disulfide bonds), hIL-11 is highly helical (57% ± 1%) and is thermally stable (melting temperature [Tm] = 90°C).[8] According to the structural model proposed by Czupryn et al,[8] IL-11 contains a four-helix bundle topology (denoted A-D) whereby methionine residue 58 (Met^{58}) and lysines (Lys^{41} and Lys^{98}) are located on the surface of the protein. Chemical modifications (alkylation or site-directed mutagenesis) of the Met^{58} residue results in a 25-fold decrease in in vitro bioactivity of rhIL-11. Chemical modification of the Lys^{41} and Lys^{98} results in a 3-fold decrease in bioactivity. rhIL-11 lacking the four carboxyl-terminal residues has a 25-fold lower bioactivity and elimination of 8 or more carboxyl-terminal residues completely abolishes activity.[9] The C-terminus of rhIL-11 is predicted to be helical and to be involved in the primary receptor binding site (site I). Important residues contributing to receptor binding in this site include Arg^{150}, His^{153}, Asp^{164}, Trp^{165}, and Arg^{168}.[8] Met^{58} is potentially involved in the receptor binding. In the region between Pro^{13} and Lys^{41}, there are a number of residues (including Pro^{13}, Glu^{16}, Leu^{17}, Leu^{22}, Arg^{25}, Leu^{28}, Thr^{31}, Arg^{32}, Leu^{34}, and Arg^{39}) that are critical for the bioactivity of IL-11 and may constitute part of a gp130 binding site (site II).[8] Lys^{41} and Lys^{98}, as well as positively charged arginine residues, which

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Table 1. Tissue/Cell Types Expressing IL-11

Tissue	Cell Type/Cell Lines	Inducers
CNS	Hippocampal neurons (H19-7)[10]	IL-1α
	Spinal motor and sympathetic neurons[10]	
	Astrocytic glioblastoma (U373, U87)[163]	IL-1β, PMA, calcium inophore
Thymus	Myeloid? (T_2)[2]	LPS
Lung	Fibroblast (MRC5, CCL202)[16]	IL-1α, TGF-$\beta_{1\text{-}2}$, PMA, RSV, Ca^{++}/calmodulin
	Epithelial (9HTE, A549)[15,17]	Rhinovirus, parainfluenza type 3, histamine (H_1)
	Muscle cells	IL-1α, TGF-β_1
Bone	Fibroblast (P-34, KM102)[5-7,71]	IL-1α, PMA, PKC
	Osteosarcoma cell lines[13,32]	IL-1α, TGF-β_1, PTH, PTHrP, cAMP, PKC
	Osteoblast[86]	IL-1α, TGF-β_1, TNF-α, PGE_2, PTH, 1α, $25(OH)_2D_3$
Connective tissues	Chondrocyte, synoviocyte[14]	IL-1β, TGF-β_1, PMA, PKC
	Vein endothelial cells[32]	IL-1α, TGF-$\beta_{1,2}$, PMA
Uterus	Fibroblast	IL-1α, PTHrP
	Trophoblast (TPA30-1)[5]	IL-1α, PMA
	Endometriotic & endometrial tissues[164,165]	
Skin	Keratinocyte[166]	
	Melanoma cell lines[167]	
	Sarcoidosis (multinucleated giant cells)[166]	
Testis	Round spermatids[10]	

are found on the exposed face of helix C, may also be involved in receptor binding site II.[9]

REGULATION OF GENE EXPRESSION

IL-11 is expressed in vivo in a wide range of normal adult murine tissues (including hematopoietic tissues) as detected by reverse transcriptase-polymerase chain reaction (RT-PCR).[10] IL-11 is detected by in situ hybridization in neurons of the central nervous system (CNS) and in developing spermatogonia of testis, where expression is developmentally regulated.[10] As summarized in Table 1, IL-11 gene expression is observed in a variety of cells of mesenchymal origin. Expression in these cells can be modulated by several inflammatory cytokines and agonists as well as hormones, either alone or synergistically. Signaling pathways involved in induction of IL-11 expression vary between different cell types. For instance, IL-11 gene expression induced by IL-1α and phorbol myristate acetate (PMA) in PU-34 cells is regulated mostly at the posttranscriptional level by increased IL-11 mRNA stabilization. IL-1α–induced IL-11 mRNA stabilization in these cells is effected through a tyrosine kinase pathway, whereas PMA-induced IL-11 mRNA stabilization is dependent on H7-sensitive serine/threonine kinases and protein kinase C (PKC) pathways. There are multiple regions (eg, 5′UTR, coding region, and 3′UTR) within the IL-11 mRNA involved in IL-1α– and PMA-induced IL-11 mRNA stabilization. In addition, the presence of ATTTA motifs in the 3′-UTR of IL-11 mRNA may function as an RNA destabilizing sequence.[4,11] Heparin, one of the extracellular matrix components that can trans-repress AP-1–mediated gene transcription, can also destabilize IL-11 mRNA after both IL-1α and PMA induction in PU-34 cells through competition for mRNA binding proteins.[12] PKC-mediated signaling events may also be involved in the induction of IL-11 in connective tissues and osteosarcoma cell lines.[13,14] Induction of IL-11 mRNA in these cells by the protein synthesis inhibitor, cyclohexamide, suggests that transcription of IL-11 is negatively regulated by protein(s) with short half-lives.[14] In contrast to BM fibroblast cells, stimulation of IL-11 gene expression by IL-1α, transforming growth factor-β1 (TGF-β1) and TGF-β2 in respiratory epithelial and fibroblast cells is likely to be transcriptionally regulated[15,16] via a pathway that is largely calmodulin-dependent and PKC-independent.[16] In addition, increased intracellular calcium and inhibition of Na+/H+ pump activity can induce IL-11 mRNA accumulation in lung fibroblast cells. The synergistic effect of histamine and TGF-β1 in induction of IL-11 in human lung fibroblasts is, to a great extent, transcriptionally regulated and dependent on H_1 receptors and a calcium/calmodulin-dependent activation pathway.[17] Thus, regulation of IL-11 expression is complex and cell/tissue specific.

HEMATOPOIETIC EFFECTS OF IL-11

Progenitor cells. IL-11 acts synergistically with other early and late acting growth factors to stimulate various stages and lineages of hematopoiesis. In synergy with IL-3,[18-20] IL-4,[19-22] IL-7, IL-12,[23-25] IL-13,[26] stem cell factor (SCF),[27] flt3 ligand,[28] and granulocyte-macrophage colony-stimulating factor (GM-CSF),[27] IL-11 stimulates the proliferation of primitive stem cells, multipotential and committed progenitor cells from various sources including cord blood,[29,30] BM,[1,30-33] and peripheral blood[34] in different culture systems.[18-20,35] This proliferation appears to be due to the entry of a quiescent (G_0) population of these cells into active cell cycle[18] as well as shortening of the cell-cycle time in some cells.[36] In combination with other cytokines present in hematopoietic microenvironment, IL-11 may increase commitment of primitive stem cells into the multilineage progenitor compartment and stimulate proliferation and differentiation of committed progenitor cells.[37] This observation is consistent with published data showing that ex vivo expansion of murine BM cells with the cytokines IL-3, IL-6, IL-

11, and SCF is associated with impaired engraftment of expanded cells in both normal and irradiated host.[38,39] However, ex vivo expansion of BM cells using IL-11 and SCF can enhance short-term engraftment potential and such expanded cells have been shown to sustain hematopoiesis during serial transplants in lethally irradiated mice.[40] In addition, chronic expression of IL-11 in hematopoietic cells via retroviral-mediated gene transfer appears to be associated with maintenance of a primitive population of cells after serial transplantation.[41] The contradictory results from these studies may be due to different cytokine combinations or concentrations used in expansion of BM cells in vitro. Although in vivo IL-11 increases the cycling rates and absolute number of myeloid progenitors in both BM and spleen of normal mice,[42] it has no effects on peripheral leukocyte counts when administered to normal rodents[43,44] and nonhuman primates.[45]

Megakaryocytopoiesis and thrombocytopoiesis. IL-11 acts synergistically with IL-3, thrombopoietin (TPO) (also termed megakaryocyte growth and development factor [MGDF]),[46,47] or SCF[48] to stimulate various stages of megakaryocytopoiesis and thrombopoiesis in both murine[49,50] and human[51-53] BM cells. In vivo treatment with IL-11 results in marked stimulation of megakaryocytopoiesis in rodents, nonhuman primates,[43-45,54] and humans[55,56] (see also below), including the production, differentiation, and maturation of megakaryocytes. In the presence of soluble c-Mpl (the receptor for TPO), megakaryocyte colony formation and acetylcholinesterease (AchE) activities induced by IL-11 alone or in combination with IL-3 or SCF are reduced.[48] Anti-TPO antiserum can also reduce IL-11–stimulated megakarocyte colony formation by 90%, whereas anti–IL-3 antiserum effects a 28% reduction in colony formation.[57] These studies suggest that IL-11 effects on megakaryocytopoiesis and thrombopoiesis may be mediated in part via TPO. Recently, Weich et al[57a] have shown that IL-11α chain mRNA was detected in purified human CD41a(+), CD14(−) megakaryocyte precursors. Further, incubation of purified cells with rhIL-11 led to rapid phosphorylation of the gp130 subunit of the IL-11 receptor, indicating direct activation of the receptor signaling subunit by IL-11. IL-11 and TPO can also synergistically stimulate the proliferation of dormant multilineage progenitors by shortening G_o, and this effect can be completely abrogated by addition of ACK2, a neutralizing antibody to c-kit, the receptor of SCF,[58] suggesting that the synergistic effects of IL-11 and TPO on multilineage cells may be mediated in part by SCF/c-*kit* interactions.

Erythropoiesis. IL-11 alone or in combination with other cytokines (IL-3, SCF, or erythropoietin [Epo]) can stimulate multiple stages of erythropoiesis using murine and human BM cells and fetal liver cells as targets.[1,32,59] The in vitro effect of IL-11 on burst-forming unit-erythroid (BFU-E) formation cannot be abrogated by antibodies against SCF, IL-3, or granulocyte-macrophage CSF (GM-CSF), suggesting a direct effect of IL-11 on human and murine erythroid progenitors.[60] In vivo studies of cytokine administration indicate that IL-11 and SCF may increase the input from a multilineage cell compartment into the erythroid lineage, whereas IL-11 and Epo may stimulate further amplification of erythroid cells. Moreover, IL-11 and SCF may lead to a redistribution of erythroid cells from BM to spleen.[61]

Myelopoiesis. IL-11 also modulates the differentiation and maturation of myeloid progenitor cells. IL-11 in combination with SCF stimulates myeloid colony formation from murine $Lin^-/Sca\ 1^+$ BM cells. These colonies are composed mostly of granulocytes and myeloid blasts. The combination of IL-11 with IL-13 or IL-4 can reduce the proportion of granulocytes and blasts in myeloid colonies, with a concomitant increase in macrophages.[26] Combination treatment with IL-11, SCF, and G-CSF in the newborn rat has been shown to significantly increase peripheral neutrophil counts.[62,63]

Lymphopoiesis. IL-11 in combination with SCF or IL-4 effectively supports the generation of B cells in primary cultures of BM cells from 5-fluorouracil (5-FU)–treated mice.[23,64,65] Similar effects have been seen with flt3/flk-2 ligand[66] using unfractionated murine fetal liver cells and with SCF and IL-7 in fractionated cells.[67] IL-11 and IL-4 can also reverse the inhibitory effect of IL-3 on early B-lymphocyte development.[68] The promotion of B-cell differentiation may be mediated by T cells.[5,69,70]

Effects on hematopoietic microenvironment. IL-11 was originally isolated from cells derived from the hematopoietic microenvironment (HM)[5-7,71,72] and may act as a paracrine or autocrine growth factor in this environment. Addition of IL-11 to human long-term BM culture (LTBMC) significantly increases the cellularity of the adherent cells, inhibits adipose accumulation in adherent cells, and leads to enhanced hematopoiesis.[73] Addition of IL-11 and SCF to bone marrow cultures derived from aplastic anemia patients significantly enhances the formation of an adherent stromal layer,[74] suggesting that IL-11 may have therapeutic value in aplastic anemia patients with defects in the HM. BM fibroblast growth can also be stimulated by the presence of megakaryocytes and the evolution of myelofibrosis is often linked with abnormal megakaryocytopoiesis. IL-11 has been shown to modulate megakaryocyte-dependent BM fibroblast stimulation.[75,76] IL-11 with other cytokines has been shown to mobilize primitive hematopoietic stem/progenitor cells both in vitro[37] and in vivo.[77] Treatment with IL-11 and SCF can enhance mobilization of long-term repopulating cells from the BM to the spleen and from the BM to the blood of splenectomized mice.[77]

NONHEMATOPOIETIC EFFECTS OF IL-11

Effects of IL-11 on epithelial cells. As mentioned above, alveolar and bronchial epithelial cells produce large amounts of IL-11. The upregulation of IL-11 production by inflammatory cytokines, respiratory syncytial virus (RSV), and retinoic acid (RA) suggests that IL-11 may play an important role in pulmonary inflammation.[15] IL-11 and IL-11Rα are also expressed in epithelial cells of the gastrointestinal (GI) tract.[78,79] In vitro studies show that IL-11 can directly interact with GI epithelial cells and reversibly inhibit proliferation of the intestinal crypt stem cell lines (IEC-6 and IEC-18).[80,81] Thus, IL-11 may be involved in the normal growth control of GI epithelial cells. IL-11–induced decrease in proliferation of these cells may due to prolongation of the G1-S phase transition which is also associated with accumulation of the

hypophosphorylated form of the retinoblastoma susceptibility gene product (pRB).[81] In addition, IL-11 has been found to enhance GI absorption of iron in rats, which does not appear to be related to changes in erythropoiesis.[82]

Osteoclastogenesis. IL-11 in combination with 1α,25-dihydroxyvitamin D 3 [1α,25(OH)$_2$ D$_3$] and parathyroid hormone (PTH) has been shown to stimulate osteoclast development and inhibit bone nodule formation in BM cultures and cocultures of BM with calvaria cells.[83,84] Osteoblasts are important regulators of osteoclast-mediated bone resorption. The requirement of the presence of stromal/osteoblastic cells in IL-11–induced osteoclast development suggests that the effect of IL-11 may be mediated through the stimulation of other factors derived from stromal/osteoblastic cells.[85] The osteoblast-dependent bone-resorptive activity of IL-11 can be inhibited by the calcitonin and cyclo-oxygenase inhibitor, indomethacin. Neutralizing antibody to IL-11 can partially negate the bone resorptive effects of PTH and block IL-1, tumor necrosis factor (TNF), and 1α,25(OH)$_2$D$_3$-induced osteoclast development.[83] IL-11 can be induced in both human and murine primary osteoblasts as well as osteoblast-like osteosarcoma cell lines (Table 1).[13] Primary osteoblasts express both IL-11Rα and gp130 mRNA, and gp130 mRNA can be upregulated by IL-1, PTH, and 1α,25(OH)$_2$D$_3$.[86] Mature osteoclasts also express IL-11Rα mRNA. These studies suggest that IL-11 is an important osteoblast-derived paracrine regulator of bone metabolism and that both bone-forming and bone-resorbing cells are potential targets of IL-11 action.[86]

Neurogenesis. Du et al[10] recently showed that IL-11 mRNA is expressed in hippocampal neuronal cells and in motor and sympathetic neurons of the spinal cord. Exogenous IL-11 stimulates the proliferation of hippocampal neuronal progenitor cells (H19-7) in a dose-dependent fashion.[10] In addition, it has been previously shown that IL-11 and several other hematopoietic growth factors are survival and/or differentiation factors for murine fetal hippocampal neuronal progenitors (MK31).[87] The production of IL-11 by alveolar and bronchial epithelial cells[15] may further suggest that IL-11 is an important survival factor for sensory and motor neurons because the subepithelial space of lung is rich in nervous innervation and IL-11 stimulates production of substance P from sympathetic neurons.[88] Previous investigators have speculated that mechanisms regulating the proliferation and differentiation of neural and hematopoietic cells may be similar.[89-94]

Other effects. IL-11 has also been shown to have other nonhematopoietic activities[1,32] such as stimulation of acute phase reactants both in vitro[95,96] and in vivo,[55] inhibition of adipogenesis,[6,73,97] induction of a febrile response,[98] and modulation of extra cellular matrix (ECM) metabolism,[14] which may have a protective effect on connective tissues or could be involved in the pathogenesis of liver fibrosis and cirrhosis.[99] In several in vitro cell culture systems, IL-11 appears to reduce pro-inflammatory cytokine expression, particularly the release of tumor necrosis factor-α (TNF-α) by monocytes/macrophages.[99a,99b]

RECEPTOR AND SIGNAL TRANSDUCTION

IL-11, like IL-6, oncostatin M (OSM), leukemia inhibitory factor (LIF), and ciliary neurotrophic factor (CNTF), uses the gp130 receptor common subunit for receptor function (Fig 1).[100] Hilton et al[101] have cloned the murine IL-11 receptor α-chain (IL-11Rα) by using a degenerate oligonucleotide probe corresponding to the conserved 5-aa motif Trp-Ser-Xaa-Trp-Ser (WSXWS) in the hematopoietin receptor family. The extracellular region of IL-11Rα shares sequence similarity to the α-chains of IL-6 and CNTF receptors (24% and 22% aa identity, respectively). The human IL-11Rα cDNA isolated by Nandurkar et al[102] predicts a 422-aa protein and shares 85% and 84% nucleotide and aa identity with the murine IL-11Rα. The extracellular region of human IL-11Rα contains a hematopoietin domain with conserved cysteine residues and the WSXWS motif. The residue 'X' differs between the human and murine receptors. There are two isoforms of human IL-11 receptor α-chain which differ in the cytoplasmic domain. One isoform of human IL-11 receptor, similar to the human IL-6 and murine IL-11 receptors, has a short cytoplasmic domain (IL-11Rα1). The other isoform, similar to the human CNTF receptor, lacks this domain (IL-11Rα2). The functional significance of differences between the two isoforms are not known yet. IL-6, CNTF, and IL-11 receptor α-chains overall share 32% identity among extracellular domains and are also structurally related. There is 42% identity between the C-terminal cytokine-receptor–like domains of IL-11Rα1 and CNTFRα.[103] The genomic structure of hIL-11Rα1 consists of 12 exons and 12 introns within a 9-kb genomic region. Human IL-11Rα gene is located on chromosome 9 band 9p13, where the CNTFR gene is also located.[104] Robb et al[105] have recently reported the structure of the murine IL-11Rα gene, which contains 14 exons. Evidence suggests the use of alternative first exons in a developmentally regulated fashion.[105] A second murine IL-11Rα–like locus (IL-11Rα2) has been reported with sequence homology to exons 2-13 of IL-11Rα1.[105] This locus appears to be present in only some strains of mice.

Binding of IL-11 ligand to either human or murine IL-11Rα occurs at low affinity and, although necessary, is not sufficient for signal transduction. The generation of a high-affinity IL-11 receptor capable of generating a biologic signal requires coexpression of the IL-11Rα and gp130.[101,102] IL-11Rα mRNA is detectable in several murine cell lines, including 3T3-L1 cells, BAd stromal cells, the embryonic carcinoma cell line PC13, and factor-dependent hematopoietic cell lines FDCP-1 and D35. A wide range of primary tissues express IL-11Rα mRNA, including hematopoietic tissues (BM, spleen, and thymus), liver, brain, heart, kidney, muscle, and salivary gland as well as cells of the GI tract.[78,101,105] Human IL-11 receptor mRNA is expressed in myeloid (K562), megakaryocytic (Mo7E), and erythroid (TF1) leukemia cell lines as well as osteosarcoma cell lines (MG-63 and Saos-2).[103]

As mentioned above, gp130 is the common subunit of the IL-6, OSM, LIF, and CNTF as well as IL-11 receptors.[106-109] Binding of IL-11 to specific cell-surface IL-11Rα receptor induces heterodimerization, tyrosine phosphorylation,[110,111] and activation of gp130.[106,108,112] The activated IL-11 receptor·gp130 complex probably activates tyrosine kinases of the Janus kinase (Jak) family (Fig 1).[113,114] IL-11 has also been shown to promote the formation of the active GTP-

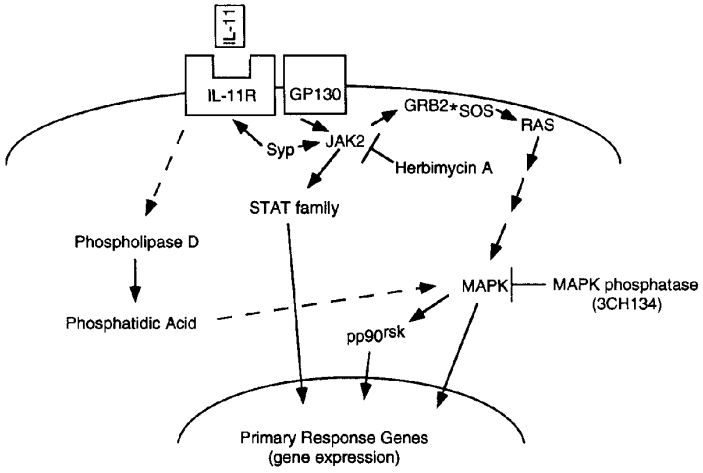

Fig 1. Possible signaling pathways mediated by IL-11.

bound form of Ras[115] and induce the tyrosine phosphorylation and activation of mitogen-activated protein kinase (MAPK),[116] a key downstream signaling target of Ras. After activation of IL-11 receptor by IL-11 binding, Jak2 forms a complex with the adapter protein, growth factor receptor binding protein 2 (Grb2), and gp130, thus bringing SOS (Son Of Sevenless) to the plasma membrane where Ras is located, hence activating Ras and initiating the Ras signaling pathway.[115] In addition to use of gp130 as a common subunit in signal transduction, the association of Jak and Ras signaling pathways on stimulation with IL-11 and similar cytokines is another unique feature of this family of cytokines. IL-11 and other cytokines using gp130 as a signal transducer can trigger the activation of MAPKs and the 85- 92-kD ribosomal protein S6 kinase (pp^{90rsk}), which is followed by activation of a set of common primary response genes (*Egr-1* or *TIS 8, TTP* or *TIS 11, Jun* B and 3CH134, which encodes a phosphatase which can inactivate MAPKs).[116,117] Src-family protein tyrosine kinases, including Fyn, Yes, and Src, may also play an important role in IL-11 signaling. Jak2 and Fyn are transiently associated with Grb2 upon stimulation with IL-11,[115] suggesting that IL-11–induced signaling in the Ras/MAPK pathway is partly through Fyn. Stimulation of 3T3-L1 cells with IL-11 results in a threefold increase in tyrosine phosphorylation of $p62^{yes}$ and a 15-fold increase in phosphorylation of $p60^{Src}$.[118]

In addition to MAPK phosphatase (3CH134), the ubiquitous tyrosine phosphatase Syp also associates with gp130 and Jak2 in response to IL-11 stimulation.[119] Herbimycin A, which is a tyrosine kinase inhibitor, can block the activation of MAPK and $pp90^{rsk}$ induced by IL-11.[116] A serine/threonine kinase inhibitor H7, which may act on signaling pathways downstream of $pp90^{rsk}$, can inhibit $pp90^{rsk}$ activity, suggesting H7-sensitive kinases are crucial in IL-11 signaling.[116,117] Lipid second messengers are also involved in IL-11 signal transduction. IL-11 treatment in 3T3-L1 cells activates phospholipase D to produce phosphatidic acid (PA). Increased levels of PA enhance tyrosine phosphorylation of MAPKs and transduce some signals in this cell line.[120] IL-11–induced phosphorylation of tyrosine kinases and H7-sensitive kinases are PKC-independent and cAMP-, cGMP-, and calcium/calmodulin-independent.[110,112] IL-11 and other cytokines sharing the signal transducing subunit, gp130, can activate acute-phase response factor (APRF) by tyrosine phosphorylation in variety of cell types. This transcriptional factor is antigenically and functionally related to members of the signal transducer and activator of transcription (STAT) family, especially STAT91. STAT91 and related proteins were originally identified as interferon-activated transcriptional factors. This suggests a central role APRF in gp130-mediated signaling.[121] IL-11 also stimulates tyrosine phosphorylation and nuclear translocation of STAT91 and a related 89-kD protein.[117] The possible signaling pathways mediated by IL-11 are summarized in Fig 1.

PRECLINICAL STUDIES

Syngeneic BM transplant (BMT) models. Administration of IL-11 accelerates recovery of megakaryopoiesis and myelopoiesis in BMT mice (Table 2).[122] Enhanced recovery of these lineages is associated with significantly decreased mortality and morbidity from lethal exogenous infection with *Pseudomonas aeruginosa* and decreased mouse-tail bleeding time.[123] BMT recipient mice treated with the combination of IL-11 and SCF show shortened periods of cytopenia in all myeloid lineages.[124] Lethally irradiated mice transplanted with syngeneic BM cells infected with a retrovirus expressing the human IL-11 cDNA demonstrate similar hematological changes as seen in BMT recipient mice treated with rhIL-11 until day 28 post BMT.[125] However, in one such study, while elevated peripheral platelet counts were sustained chronically, no changes in peripheral erythrocyte or leukocyte counts were observed long term despite a greater than 20-fold increase in splenic myeloid progenitor content. Two of 20 secondary recipients of BM cells transduced with a retrovirus expressing hIL-11 cDNA developed myeloid leukemia. All mice showed systemic effects of chronic IL-11 exposure (Table 2).[126,127] A recent study has shown that ectopic expression of murine IL-11 via a retrovirus vector

Table 2. Effects of IL-11 on Cytoablative Preclinical Models

Models	Peripheral Blood	Bone Marrow	Spleen	Thymus	GI Tract	Other
BMT[122-127]	WBC↑, N↑ Plt↑ Reticulocyte↑ *Pseudomonas aeruginosa* LD_{50}↑ Tail bleeding time↓	CFU-mix↑ CFU-GM↑ CFU-MK↑ Cellularity↑	CFU-mix CFU-GM↑ CFU-MK↑ Cellularity↑			Myeloid leukemia Body weight↓ Hyperactive state Thymic atrophy Inflammation Acute-phase protein↑
Sublethal[122,128]	WBC↑ Plt↑		Cellularity↑ T- & B-cell function	Cellularity↑ T- & B-cell function↑		Pneumonitis
Chemotherapy[42,66,129-133]	WBC↑, N↑ Plt↑ Hct↑ Pancytopenia↓ SRBC-specific Ab↑	Cellularity↑ CFU-mix↑ CFU-GM↑ BFU-E↑	tCellularity↑ SRBC-specific plaque Forming cells↑ CFU-mix, GM, BFU-E↑		↓Frequency, severity and duration of oral mucositis	↑Survival
Chemoradiation therapy[79,134]	Late recovery of Plt & Hct Mild ↑Plt at early stage postirradiation	BFU-E↑ CFU-GM↑ CFU-MK↑ at day 15 & 30 postirradiation	BFU-E, CFU-GM & CFU-MK↑ at day 15 postirradiation		↑Mitotic index & $PCNA^+$ cells in crypts ↓Apoptosis	↑Recovery of spermatogenesis ↑Survival

Abbreviations: CFU-mix, colony-forming unit-mix; CFU-GM, colony-forming unit-granulocyte/macrophage; CFU-MK, colony-forming unit-megakaryocyte; WBC, white blood cells; N, neutrophils; Plt, platelets; Hct, hematocrit; SRBC, sheep red blood cell; PCNA, proliferating cell nuclear antigen.

accelerated recovery of platelets and leukocytes (neutrophils) in secondary and tertiary BMT mice. This study also suggests that IL-11 expression in vivo may enhance maintenance of primitive hematopoietic stem cells.[41]

Sublethal radiation (non-BMT) models. In contrast to the effects in BMT models, IL-11 treatment has little effect on progenitor compartments in sublethally (600 cGy) irradiated mice.[122] IL-11 treatment was shown to restore thymus and spleen cell numbers as well as T- and B-cell mitogen responsiveness in mice exposed to 200 cGy irradiation (Table 2). Sublethal irradiated dogs (200 cGy) treated with IL-11 show a modest trend toward faster platelet recovery. Some of the dogs in this study demonstrated pneumonitis, the etiology of which is unclear.[128]

Chemotherapy models. Chemotherapy is often associated with blood cytopenias and immunosuppression as well as GI mucosal damage. IL-11 treatment significantly reduces chemotherapy related morbidity and mortality[129-133] and is associated with accelerated recovery of both hematopoiesis[42,132] and the immune response[42,70] in different chemotherapy preclinical models (Table 2). Mortality associated with repeated doses of 5-FU is abrogated by pretreatment with IL-11 and SCF, but not by infusion with BM cells, suggesting that in this model IL-11 and SCF pretreatment may protect tissues other than hematopoietic tissues adversely affected by chemotherapy.[129] In a hamster model of oral mucositis, IL-11 decreases the frequency, severity, and duration of oral mucositis in a dose-dependent fashion[131,133] with little changes on BM cellularity, strengthening the suggestion that the protective mechanism of IL-11 on mucositis is due, at least in part, to effects on epithelial and/or connective tissues.[133]

Combined chemo-/radiation therapy models. IL-11 administration markedly decreases morbidity and mortality due to sepsis by endogenous gut organisms[79] and accelerates recovery of spermatogenesis[10] in mice treated with combined chemo-/radiation therapy (5-FU and sublethal irradiation). The increased survival is associated with increased proliferation of crypt cells and decreased apoptosis of villous/crypt cells.[134] The seemingly contradictory effects of IL-11 on GI crypt cell proliferation seen in in vitro[78,80] and in vivo[79] studies may be due to distinctly different effects on damaged versus undamaged cell populations: inhibition of proliferation before damage (seen in in vitro cell lines) and stimulation of proliferation post damage (seen in in vivo models of gut cell damage). This explanation is supported by the finding that pretreatment of mice with IL-11 followed by irradiation is associated with significant increases in the survival of intestinal crypt stem cells.[135] In addition, recent studies show pretreatment of mice with IL-11 significantly reduces ischemia/reperfusion-induced small-bowel injury.[136] The effect of IL-11 on combined chemo-/radiation therapy–induced gut mucosal damage may prove to be important in clinical use in cancer chemotherapy and BM transplant protocols in the future. The effects of IL-11 on cytoablative preclinical models are summarized in Table 2.

Other GI disease models. Acute colitis caused by chemical damage and chronic inflammatory bowel disease in transgenic animals expressing human HLA-B27 and β2-microglobulin are improved at both the gross and microscopic level by administration of IL-11.[131] IL-11 treatment has proliferative effects on intestinal mucosa in mice after ischemic bowel necrosis,[136] in a murine burn model,[137] and in a rat short-bowel model.[138] In all of these models, significantly increased survival rates are seen in mice treated with IL-11. IL-11 treatment also increases peripheral lymphocyte counts and decreases enteric bacterial translocation in both bowel ischemia and systemic burn models.

Sepsis models. Pretreatment with IL-11 significantly reduces mortality in a murine model of toxic shock syndrome[139] and in experimental group B streptococcal (GBS) sepsis in neonatal rats.[140] Endogenous IL-11 may play a role in the pathophysiologic response of neonatal animals to bacterial sepsis and associated thrombocytopenia.[140] In a rabbit model of endotoxemia, IL-11 treatment prevents hypotension and decreases GI mucosal damage induced by lipopoly-

saccharide (LPS).[141] The anti-inflammatory effects of IL-11 on both murine and rabbit models of endotoxemia appears to be due to inhibition of the production of proinflammatory mediators through effects on macrophages.[142]

IL-11 AND DISEASES

IL-11 acts as a synergistic factor with IL-3, GM-CSF, and SCF to stimulate proliferation of human primary leukemia cells, myeloid leukemia cell lines,[143,144] megakaryoblastic cell lines,[145] and erythroleukemic cell lines[108] and to stimulate leukemic blast colony formation.[143,144] IL-11 mRNA expression in leukemic cells and inhibition of leukemic cell growth by IL-11 antisense oligonucleotides suggest that IL-11 may function as an autocrine growth factor in leukemic cell lines.[144,145] Although IL-11 stimulates the proliferation of murine plasmacytoma cells[5,146] and murine hybridoma cells,[147-149] the effect of IL-11 on the growth of human myeloma/plasmacytoma cells is controversial. IL-11 has no effect on the growth of freshly isolated human plasmacytoma cells.[146,150] However, IL-11 can stimulate proliferation in two of eight human myeloma cell lines tested so far.[146,151] As expected, anti-gp130 monoclonal antibodies can inhibit growth stimulation by IL-11 in human myeloma cell lines.[152] The plasmacytoma growth inhibitor restrictin-P (also called activin A, follicle-stimulating hormone releasing protein, or erythroid differentiation factor), another growth regulatory protein derived from BM stromal, can inhibit the growth of IL-11–stimulated murine hybridoma cells.[153]

HUMAN STUDIES AND CLINICAL TRIALS

IL-11 has now been evaluated in several human clinical trials. In the initial phase I trial, women with advanced-stage breast cancer undergoing high-dose chemotherapy were treated with increasing doses of IL-11 (up to 100 μg/kg/d) both before therapy and after each of four cycles of combined chemotherapy. IL-11 administration was associated with a dose-dependent trend toward increased platelet counts, and patients receiving rhIL-11 at doses >25 μg/kg/d showed attenuated postchemotherapy thrombocytopenia after the first and second cycles.[55] Increased peripheral platelet counts were associate with both stimulation of platelet production and megakaryocyte maturation, as evidenced by increased numbers of BM colony-forming unit-megakaryocyte (CFU-MK), increased megakaryocyte numbers, and higher megakaryocyte ploidy.[56] In contrast to effects seen in various preclinic studies, IL-11 treatment in this trial had no significant effect on leukopenia or neutropenia due to chemotherapy.[55] However, IL-11 treatment was associated with increased BM cellularity, and increased numbers and cycling of immature erythroid and myeloid precusors.[56]

IL-11 treatment in these patients was well tolerated at doses of 10 to 50 μg/kg/d. The most common side effect noted was a reversible anemia. The anemia was non–dose-related and decreases of $\approx$20% in hematocrits, possibly due to increased plasma volume, were seen.[55,154] Other reversible side effects included arthralgias, myalgias, fatigue, nausea, headache, and edema. Unlike many other cytokines, IL-11 treatment was not associated with an increased incidence of fever. IL-11 administration increased the plasma concentrations of acute-phase reactants, including C-reactive protein, fibrinogen, and haptoglobin at all doses.[55]

In several phase I/II trials, IL-11 has also been well tolerated in doses up to 50 μg/kg/d and appears to be a promising agent for accelerating hematopoietic recovery after multiple cancer therapies. The combined administration of IL-11 with G-CSF (5 μg/kg/d) in breast cancer patients receiving high-dose cyclophosphamide, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), and thiotepa followed by autologous BMT effectively accelerates both peripheral neutrophil and platelet recoveries.[155] In a phase I/II trial in children with solid tumors or lymphoma, IL-11 (50 μg/kg/d) and G-CSF (10 μg/kg/d) administration after ICE (ifosfamide, carboplatin, and etoposide) chemotherapy appears to decrease the median number of platelet transfusions required (12 *v* 2), and reduces the days to recovery of both neutrophils (21 *v* 17.5 days) and platelets (27 *v* 22 days) when compared to ICE + G-CSF alone.[156] Preliminary results from both trials cited above have not been reported in full at this point and it is not clear whether these differences are significant.

A multicenter, randomized, placebo-controlled IL-11 phase II clinical trial has been conducted in 93 cancer patients who had received at least one platelet transfusion during a prior chemotherapy cycle (secondary prophylaxis design). These patients were given an additional cycle of the same chemotherapy without dose reduction and were randomized to receive either rhIL-11 (at a dose of 25 or 50 μg/kg) or placebo. The patients treated with rhIL-11 in this phase II study were less likely to require platelet transfusions than the patients receiving placebo. For the patients treated with IL-11 at 25 μg/kg and 50 μg/kg, 30% (8 of 27) required no platelet transfusions compared to 1 of 27 patients treated with placebo. This difference was statistically significant ($P < .05$). The median number of platelet transfusions required among the groups treated with 50 μg/kg, 25 μg/kg, and placebo was 1, 2, and 3, respectively. The profile of side effects was similar to that seen in phase I studies. Most side effects were mild to moderate in severity and were reversible after IL-11 treatment was discontinued.[157,158] Based on observations of the potent effects of IL-11 in models of gut damage, a major advantage of IL-11 may be the simultaneous effects of the cytokine on both BM and GI toxicities of chemotherapy and irradiation. A dose-escalating phase I/II randomized placebo-controlled human study examining the effects of IL-11 in patients with Crohn's disease has recently been completed.[168] Based on the results of this trial, additional trials in Crohn's disease and in chemotherapy-induced mucositis are anticipated (Genetics Institute, personal communication, James Kaye, October 1996).

The recent cloning of the ligand for c-mpl[159-162] provides another, and potentially very useful, therapeutic approach to thrombocytopenic states. Early trials with TPO (also termed MGDF) appear promising and it will require multiple trials in various pathologic conditions to determine optimal cytokine combinations to enhance recovery of hematopoietic lineages with the least side effects. At the present time it would appear that IL-11 will be a useful thrombopoietin and may be uniquely useful in stimulating the recovery of the BM and the GI tract simultaneously after therapy-induced damage.

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Review

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Interleukin-6 and interleukin-11: same same but different

Abstract: The pleiotropic physiological functions of interleukin (IL-)6 type cytokines range from embryonic development and tissue homoeostasis to neuronal development and T cell differentiation. In contrast, imbalance of the well-controlled cytokine signaling network leads to chronic inflammatory diseases and cancer. IL-6 and IL-11 both signal through a homodimer of the ubiquitously expressed β-receptor glycoprotein 130 (gp130). Specificity is gained through an individual IL-6/IL-11 α-receptor, which does not directly participate in signal transduction, although the initial cytokine binding event to the α-receptor leads to the final complex formation with the β-receptors. Both cytokines activate the same downstream signaling pathways, which are predominantly the mitogen-activated protein kinase (MAPK)-cascade and the Janus kinase/signal transducer and activator of transcription (Jak/STAT) pathway. However, recent studies have highlighted divergent roles of the two related cytokines. Here, we will discuss how the biochemical similarities are translated into unique and non-redundant functions of IL-6 and IL-11 *in vivo* and illustrate strategies for cytokine-specific therapeutic intervention.

Keywords: gp130; IL-11 receptor; IL-6 receptor; interleukin 11; interleukin 6; STAT3.

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Introduction: IL-6 family of cytokines in health and disease

Interleukin (IL-)6 type cytokines play integral roles in numerous physiologic and pathophysiologic processes. On one hand they are engaged in development and tissue homeostasis, and on the other hand critically involved in immunity, infection and inflammation. Their partly overlapping and redundant spectrum of actions, ranging from specific functions during embryonic development or hematopoiesis, is accompanied by distinct functions that can be pinpointed to one specific family member and cannot be fulfilled by the other members.

The Interleukin (IL-)6 family of cytokines consists of IL-6, IL-11, IL-27, IL-30, IL-31, oncostatin M (OSM), leukemia inhibitory factor (LIF), cardiotrophin-1 (CT-1), cardiotrophin-like cytokine (CLC), ciliary neurotrophic factor (CNTF) and neuropoeitin (NP-1). With the exception of IL-31, all of these cytokines engage a homo- or heterodimer of the ubiquitously expressed signal-transducing β-receptor glycoprotein 130 (gp130). Recently, the heterodimeric cytokine IL-35, which is composed of EBI3 (also a part of IL-27) and p35 (also a part of IL-12), has also been shown to signal via gp130, being therefore a shared member of the IL-6 and IL-12 cytokine families (Collison et al., 2012). Formation of the individual cytokine signaling complexes on the cellular membrane triggers activation of the major downstream signaling pathways Janus kinase/signal transducer and activator of transcription (Jak/STAT), the mitogen-activated protein kinase (MAPK) pathway and (to a minor extent) the phosphatidyl-inositol-3-kinase (PI3K)-cascade. In this review, we will focus on the two family members IL-6 and IL-11, which both signal via a gp130 homodimer [for detailed information concerning signal transduction of all IL-6 family members see for example (Heinrich et al., 2003; Eulenfeld et al., 2012; Garbers et al., 2012; Mohr et al., 2012)].

The IL-6 family members IL-6 and IL-11 are four-helical bundle cytokines with an up-up-down-down topology. Both proteins are transcribed with a canonical signal peptide that ensures efficient secretion from cells. The mature form of IL-6 consists of 183 amino acids, whereas IL-11 encodes a mature protein of 178 amino acid residues. IL-6 and IL-11 share little sequence homology (about 22%) even though they both transduce signals via a homodimer of two gp130 β-receptor molecules, whereas all other IL-6

type cytokines bind to gp130-LIFR/OSMR/WSX-1 heterodimers or in the case of IL-31 to Gp130-like receptor (GPL) and OSMR (Bazan, 1989, 1990). IL-6 and IL-11 cannot bind to the ubiquitously expressed gp130 in the absence of a specificity factor, the so-called α-receptors (IL-6R and IL-11R) (Figure 1). Accordingly, expression of the IL-6R and IL-11R determines whether IL-6, IL-11 or both can activate a cell. Like IL-6 and IL-11, the extracellular domains of IL-11R and IL-6R show low sequence identity of about 24% (Curtis et al., 1997). Inter-species comparison of the IL-6R and IL-11R between mice and humans however reveals a sequence identity of about 82% and 52%, respectively. IL-6 signaling shows a remarkable species-specificity, as human IL-6 can activate both the murine and the human IL-6R, whereas murine IL-6 acts solely on the murine IL-6R (Jostock et al., 2001; Garbers et al., 2011). Species-specificity for IL-11/IL-11R has not been analyzed thus far. Crystal structures of the cytokine/receptor complex have been solved for IL-6 in complex with IL-6R and gp130 (Chow et al., 2001; Boulanger et al., 2003), and the signaling complexes formed with IL-6/IL-6R and IL-11/IL-11R appear to have similar conformations and dynamics in the membrane-proximal regions (Matadeen et al., 2007; Xu et al., 2010; Lupardus et al., 2011). Site I of IL-6 and IL-11 are binding sites towards IL-6R and IL-11R, respectively, whereas site II and site III of IL-6 and IL-11 are binding sites towards gp130.

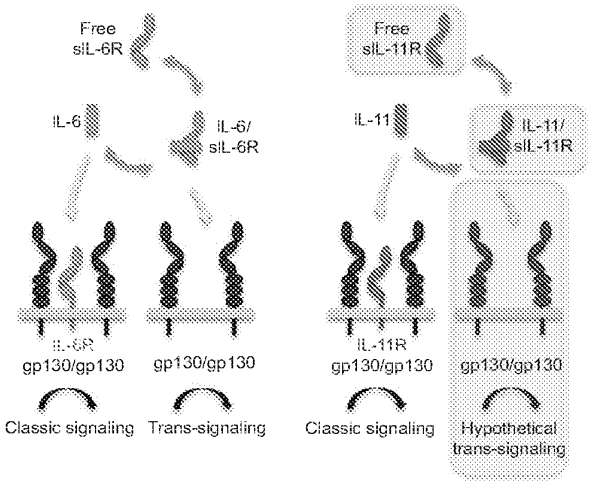

Figure 1 Receptor composition for IL-6 and IL-11 signaling via the β-receptor subunit gp130.IL-6 has two mechanisms of cellular activation.
IL-6 classic signaling requires IL-6 binding to a membrane-associated receptor (IL-6R). In trans-signaling, IL-6 activates cells lacking membrane-bound IL-6R through an IL-6/sIL-6R complex, which binds directly to the ubiquitously expressed gp130. To date, only IL-11 classic but not trans-signaling was described.

The IL-6R is mainly expressed on hepatocytes and some leukocytes, including macrophages, monocytes, neutrophils, B- and T cells (Chalaris et al., 2011). IL-11R has been found on lymphocytes, B-cells, macrophages, endothelial cells, hematopoetic cells and osteoclasts (Figure 2) (Putoczki and Ernst, 2010). This list is, however, certainly far from being complete, and the future will hopefully bring a more comprehensive list of cells that are responsive to IL-6 and IL-11. For instance, alpha cells of the pancreas have been shown to express IL-6R and IL-6 controls expression alpha cell mass and thereby glucagon level and glucose homeostasis (Ellingsgaard et al., 2008). Ultimately, this shows that some cells are responsive to IL-6 and IL-11 and some are exclusively targeted by IL-6 or IL-11. Detailed analysis of how cells shape their receptor expression profiles are missing, but it is likely that cells can modulate their ability to respond to the cytokines IL-6 and IL-11. Two papers have shown consistently that the synthetic glucocorticoid dexamethasone is capable of up-regulating IL-6R mRNA (Rose-John et al., 1990; Snyers et al., 1990), and this was recently also confirmed on the protein level (Ladenburger et al., 2010). Interestingly, Interleukin 2 (IL-2) has been shown to regulate TH17 differentiation by inducing down-regulation of the IL-6R and gp130 on T cells, therefore limiting the ability of IL-6 to induce TH17 cells (Liao et al., 2011). Furthermore, activated $CD4^+$ T cells were shown to lose membrane-bound IL-6R via a process called ectodomain shedding mediated by ADAM proteases (Briso et al., 2008; Scheller et al., 2011a). Mechanisms that

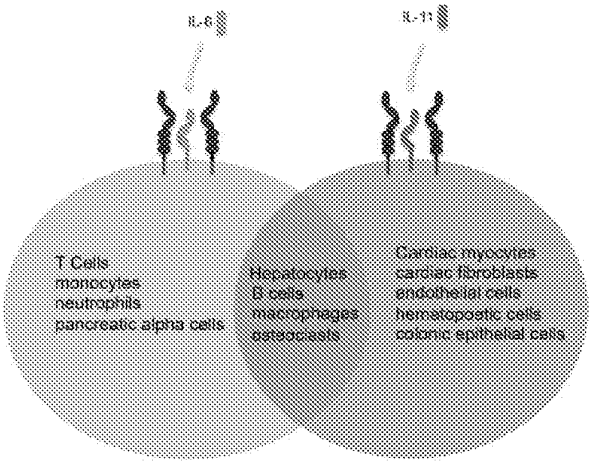

Figure 2 Distinct and combined cellular expression of IL-6R and IL-11R.
The Venn diagram illustrates cell types mentioned in this review that either only express IL-6R (left part), only IL-11R (right part), or are known to express IL-6R as well as IL-11R (overlapping middle part).