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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 May 2009 (14.05.2009)

PCT

(10) International Publication Number
WO 2009/059425 A1

(51) International Patent Classification:
A61K 31/575 (2006.01) *A61K 45/00* (2006.01)
A61K 31/122 (2006.01) *A61K 47/48* (2006.01)
A61K 31/202 (2006.01) *A61K 48/00* (2006.01)
A61K 31/381 (2006.01) *A61K 9/72* (2006.01)
A61K 31/42 (2006.01) *A61P 11/00* (2006.01)
A61K 31/7105 (2006.01) *C12Q 1/02* (2006.01)
A61K 38/17 (2006.01) *A61K 9/00* (2006.01)

(21) International Application Number:
PCT/CA2008/001971

(22) International Filing Date:
7 November 2008 (07.11.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/996,235 7 November 2007 (07.11.2007) US

(71) Applicants *(for all designated States except US)*: **THE UNIVERSITY OF BRITISH COLUMBIA** [CA/CA]; University - Industry Liaison Office, #103 - 6190 Agronomy Road, Vancouver, British Columbia V6T 1Z3 (CA). **UNIVERSITY OF WESTERN AUSTRALIA** [AU/AU]; 35 Stirling Highway, Crawley, Western Australia 6009 (AU). **LUDWIG INSTITUTE FOR CANCER RESEARCH LTD** [US/US]; 605 Third Avenue, New York, New York 10158 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **KNIGHT, Darryl Andrew** [AU/CA]; 1130 East 16th Avenue, Vancouver, British Columbia V5T 2W3 (CA). **MUTSAERS, Steven Eugene** [AU/AU]; c/o Lung Institute of Western Australia, Ground floor E block, Sir Charles Gairdner Hospital, Hospital Avenue, Nedlands, Western Australia 6009 (AU). **O'DONOGHUE, Robert Joseph James** [AU/AU]; Unit 1, 53 Kelvin Street, Maylands, Western Australia 6051 (AU). **ERNST, Matthias Rober Walter** [CH/AU]; c/o Ludwig Institute for Cancer Research Ltd, Melbourne Branch, PO Box 2008, Royal Melbourne Hospital, Victoria 3050 (AU).

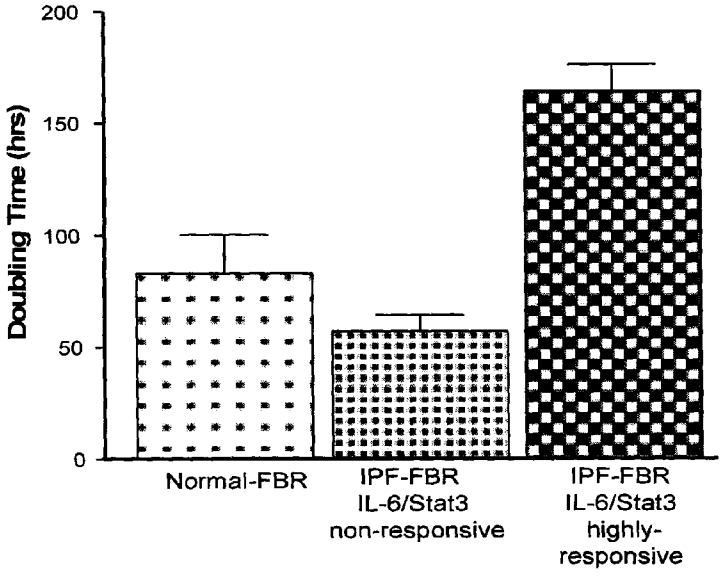
(74) Agent: **MACINS, Andris D.**; Smart & Biggar, Box 11560, Vancouver Centre, 2200-650 West Georgia Street, Vancouver, British Columbia V6B 4N8 (CA).

(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, BR, BW, BY, BZ, CA,

[Continued on next page]

(54) Title: STAT3 INHIBITORS FOR THE TREATMENT OF FIBROSIS

FIGURE 8



(57) Abstract: A method of medical treatment or prevention of an interstitial lung disease (ILD) in a subject in need thereof, the method including administering a therapeutically effective amount of a signal transducer and activator of transcription 3 (STAT3) inhibitor to the subject is provided. In other aspects uses of STAT3 inhibitors for treatment and/or prevention of an ILD, or for preparation of medicaments for treatment or prevention of an ILD are provided. The method or use may further include coupling the STAT3 inhibitor to a targeting moiety.

WO 2009/059425 A1

CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, 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, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

STAT3 INHIBITORS FOR THE TREATMENT OF FIBROSIS

Technical Field

This invention relates to the field of fibrotic pathology. More particularly to the treatment of fibrotic pathology by signal transducer and activator of transcription 3 (STAT3) inhibition.

Background

Signal transducer and activator of transcription 3 (STAT3) is a transcription factor that is involved in diverse processes, such as cell proliferation and migration, inflammation, immune response and cell survival (Lim *et al.* 2006). The STAT family of transcription factors transduce extracellular signals from cytokines such as interleukin-6 (IL-6) or interferons, and from growth factors such as platelet-derived growth factor (PDGF) or epidermal growth factor (EGF). STAT3 is activated by phosphorylation of a conserved tyrosine residue enables the dimerization of the protein – either as a homodimer, or as a heterodimer with STAT1. Dimerized STAT3 permits translocation to the nucleus where it is able to bind to specific DNA sequences and regulate the transcription of a variety of genes (Hodge *et al.* 2005). STAT3 is an essential mammalian gene, whereby a total STAT3 knockout in mice results in embryonic lethality (Takeda *et al.* 1997).

Downregulation of STAT3 has been suggested for the treatment of various types of cancer, particularly those characterized by having abnormally high levels of activated STAT3. For instance, it has been reported that 60% of breast tumours contain persistently activated STAT3 (Dechow *et al.* 2004). STAT3 may participate in oncogenesis by stimulating cell proliferation, promoting angiogenesis, and/or conferring resistance to apoptosis. WO 2002/078617 discloses methods for treatment of cancer using a STAT3 inhibitor. Other Inhibitors of STAT3 are known in the art (for example, see WO 2006/091837; Song *et al.* (2005); Protein Inhibitor of Activated STAT3 (PIAS3) Chung *et al.* (1997); and peptidomimetic inhibitors Coleman *et al.* (2005)).

STAT3 has been associated with renal fibrosis (Huang J-S. *et al.* (1999)) and liver fibrosis (Si HF. *et al.* (2007); and Stärkel P. *et al.* (2007)). Furthermore, the JAK-STAT pathway has been associated with human airway smooth muscle cell remodeling in asthmatic patients (Simon AR. *et al.* (2002)). Wu SH. *et al.* (2006) describe connective

tissue growth factor (CTGF) and STAT3 in normal human lung fibroblasts. Walters DM. *et al.* (2005) describe STAT3 phosphorylation in mouse fibroblasts from STAT1 deficient mice. Studies have demonstrated a possible role for STAT3 mediated signaling in the pathogenesis of fibrotic diseases characterized by excessive fibrosis (Moodley *et al.*, 2003a, b). The pathology of fibrotic diseases is complex and the exact role of STAT3 mediated signaling in the onset and progression of fibrotic diseases is unclear.

Pulmonary fibrosis is the end-point of a heterogeneous group of disorders classified as interstitial lung diseases (ILD) and is characterized by excessive extracellular matrix accumulation leading to progressive organ dysfunction and premature death of the individual. Idiopathic pulmonary fibrosis (IPF) is the most aggressive of the ILDs (Pardo and Selman 2002), with the progression of IPF intimately associated with regions of fibroblast accumulation referred to as 'fibroblastic foci'. The extent of these foci is a reliable indicator of disease severity and mortality. Ultrastructural and histochemical studies have shown that a large number of fibroblasts within these foci express α-smooth muscle actin (α-SMA) (Selman *et al.* 2001) and are termed myofibroblasts. These differentiated fibroblasts are found in greatest numbers in regions of dense fibrosis in the lung and express higher levels of collagen. Currently, the only effective treatment for this disease is lung transplant. As such, strategies that modulate the formation and/or function of these cells may be useful for treatment of IPF (Ramos *et al.* 2001).

Conflicting literature surrounds the question of whether IPF-fibroblasts proliferate faster than normal-fibroblasts, or are resistant to apoptosis. It has been reported that fibroblasts derived from IPF lungs proliferate faster than cells derived from normal lung tissue (Jordana *et al.* 1988; Moodley *et al.* 2003b). In contrast, others have shown that the growth rate of IPF-fibroblasts was significantly slower than normal-fibroblasts (Ramos *et al.* 2001).

Interleukin-6 (IL-6) is released by a variety of cells, including fibroblasts, and has been shown to mediate many inflammatory processes in the lung. IL-6 has been demonstrated to have an inhibitory effect on the proliferation of normal lung fibroblasts, and this effect appears to be mediated in part by STAT3 (Moodley *et al.* 2003b). On the other hand, IL-6 increased the proliferation of fibroblasts isolated from fibrotic lung tissue, an effect that was associated with decreased activation of STAT3 (Moodley *et al.* 2003b). Similarly, IL-6 had a differential effect on apoptosis of fibroblasts from normal and fibrotic lung tissue – in normal fibroblasts, IL-6 was pro-apoptotic, whereas IL-6 protected

IPF fibroblasts from Fas-Ligand induced apoptosis (Moodley *et al.* 2003a). Thus, there is conflict in the literature in terms of the role of STAT3 in cytokine stimulated growth and apoptosis of fibroblasts during the development and progression of pulmonary fibrosis.

It would appear that cytokine signaling has a role in the pathology of fibrotic diseases, as does STAT3. However, the specific molecular events that regulate fibrosis in different fibrotic diseases are not clear. Furthermore, based on the available information, prediction of the clinical implications of reduced or enhanced expression or function of STAT3 is difficult (Gao H. and Ward PA. 2007).

Summary

This invention is based, in part, on the surprising discovery of different fibroblast phenotypes from fibrotic lungs, and that elevated levels of activated STAT3 are found in the slow growing, hypertrophic fibroblasts derived from fibrotic lung tissue. Increased STAT3 activity was found to correlate with an increase in collagen α1 expression and collagen deposition in lung tissue, and increased collagen α1 promoter activity in cultured lung fibroblasts.

In accordance with one aspect of the invention, there is provided a method of preventing or treating an interstitial lung disease (ILD) in a subject in need thereof, the method including administering to the subject a signal transducer and activator of transcription 3 (STAT3) inhibitor.

In accordance with another aspect of the invention, there is provided a use of a STAT3 inhibitor in the manufacture of a medicament for the prevention or treatment of an ILD in a subject in need thereof.

In accordance with another aspect of the invention, there is provided a use of a STAT3 inhibitor for the prevention or treatment of an ILD in a subject in need thereof.

In accordance with another aspect of the invention, there is provided a use of a pharmaceutical composition including a STAT3 inhibitor for the prevention or treatment of an ILD in a subject in need thereof.

In accordance with another aspect of the invention, there is provided a commercial package including: (a) a pharmaceutical composition including: (i) STAT3 inhibitor; and (ii) a pharmaceutically acceptable carrier; and (b) instructions for the use thereof for treating an ILD.

The STAT3 inhibitor may be selected from one or more of: a STAT3 peptidomimetic; Protein Inhibitor of Activated STAT3 (PIAS3); a STAT3 antisense RNA; a STAT3 siRNA; Lipoxin A_4 (LXA_4); LY294002; STA-21; Leflunomide; 6-nitro-1-benzothiophene 1,1-dioxide (CID: 2779853); Cucurbitacin D; Tetrahydro- Cucurbitacin I; NSC628869; NSC94743; NSC49451; NSC521777; NSC106399; and NSC135075; analogues thereof; variants thereof; pharmaceutically acceptable salts thereof; a pro-drugs thereof; a metabolites thereof; and a pharmaceutical composition including one or more of the above. The antisense RNA may correspond to one or more of: SEQ ID NO:1; SEQ ID NO:11 and SEQ ID NO:12. Alternatively, the antisense RNA may be targeted to any 8-30 bases found in SEQ ID NOs:2-8 or may be targeted to SEQ ID NO:9 and SEQ ID NO:10. The STAT3 inhibitor may be STA-21. The STAT3 inhibitor may be PIAS3. The STAT3 inhibitor may be LXA4. The STAT3 inhibitor may be LY294002. The STAT3 inhibitor may be 6-nitro-1-benzothiophene 1,1-dioxide. The STAT3 inhibitor may be NSC94743. The STAT3 inhibitor may be NSC628869. The STAT3 inhibitor may be NSC49451. The STAT3 inhibitor may be NSC521777. The STAT3 inhibitor may be NSC106399. The STAT3 inhibitor may be NSC135075. The STAT3 inhibitor may be Leflunomide. The STAT3 inhibitor may be Cucurbitacin D. The STAT3 inhibitor may be Tetrahydro-Cucurbitacin I. The STAT3 inhibitor may be a STAT3 peptidomimetic. The STAT3 inhibitor may be any combination of the above STAT3 inhibitors. The STAT3 inhibitor may be further targeted to slow growing, differentiated, hypertrophic fibroblasts in fibrotic lung tissue. The STAT3 inhibitor may be further coupled to a targeting moiety. The targeting moiety may be an antibody having specificity for one or more of the following: fibronectin (FN1); collagen 1α1 (COL1A1); α-smooth muscle actin (α-SMA); connective tissue growth factor (CTFG); transforming growth factor beta 1 (TGFB1); and interleukin-6 (IL-6). The antibody may be humanized. The STAT3 inhibitor may be further coupled to a cell uptake moiety. The targeting moiety may act as a cell uptake moiety. The STAT3 inhibitor may be formulated for inhalation. The STAT3 inhibitor may be formulated for administration by injection. The STAT3 inhibitor may be formulated for oral administration. The STAT3 inhibitor may be formulated for topical administration. The subject may be a human. The ILD may be fibrotic lung disease. The ILD may be selected from one or more of: idiopathic pulmonary fibrosis (IPF); pulmonary fibrosis (PF); acute lung injury; hypersensitivity pneumonitis; and drug-induced lung fibrosis. The ILD may be IPF.

In accordance with another aspect of the invention, there is provided a method for screening for or identifying a test compound that inhibits the production of collagen in a fibroblast, the method including administering the test compound or test compounds to fibroblasts and assaying for decreased expression or activity of collagen or the collagen α1 promoter.

In accordance with another aspect of the invention, there is provided a method for screening for or identifying a test compound that inhibits α-SMA in a fibroblast, the method including administering the test compound or test compounds to fibroblasts and assaying for decreased expression or activity of α-SMA.

In accordance with another aspect of the invention, there is provided a method for screening for or identifying a test compound that inhibits STAT3 expression or activity in a fibroblast, the method including administering the test compound or test compounds to fibroblasts and assaying for decreased expression or activity of STAT3.

The screening may be carried out on slow growing, differentiated, hypertrophic fibroblasts obtained from fibrotic lung tissue. The test compounds may be administered to an ILD or IPF animal model. Furthermore, Thy-1 levels may be monitored as an indication of fibroblast cell response to a test compound.

Brief Description of the Drawings

Figure 1. STAT3 activation in response to IL-6 stimulation in the lung derived fibroblasts from patients with Idiopathic Pulmonary Fibrosis (IPF). Fibroblasts derived from lung tissue samples of patients with IPF and normal controls were stimulated with human recombinant IL-6 (100 ng/ml) for indicated time periods. Bar graph demonstrates different levels of IL-6-induced STAT3 activation in fast and slow growing fibroblasts obtained from the lung tissue samples of patients with IPF in comparison with fibroblasts from normal controls. The X-axis shows the different sample time points, while the Y-axis shows the level of activated STAT3 as compared to untreated controls (fold increase).

Figure 2. Human adult lung derived fibroblasts transfected with constitutively active STAT3 (STAT3C) construct acquire a phenotype of slow growing cells characterized by hyper-activated STAT3 response to gp130 stimulation. Panels depict phase contrast micrographs obtained from cells in primary cultures and demonstrate a hypertrophic phenotype acquired by STAT3C transfected cells (B), which is similar to

slow growing fibroblasts (D). In contrast, fibroblasts transfected with empty vector (C) (control for STAT3C transfected cells) look like fast growing cells (A) with normal and/or decreased responses to gp130 stimulation (original magnification X250).

Figure 3. Collagen α1 gene expression and total collagen synthesis are increased in the lung tissue of transgenic mice with hyper-activated STAT3 after bleomycin challenge. (A) shows total lung collagen in the lung tissues from wild-type (WT), STAT3 defective (ΔSTAT3), hyper-activated STAT3 (gp130757F), and STAT3 activation compromised (gp130757FxSTAT3+/-) animals after bleomycin exposure. The X-axis shows each of the different animal genotypes, while the Y-axis displays the total lung collagen, estimated by measurement of hydroxyproline (Hyp) levels per 104 lung cells. (B) shows collagen α1 mRNA expression and collagen production from wild-type (WT), STAT3 defective (ΔSTAT3), hyper-activated STAT3 (gp130757F), and STAT3 activation compromised (gp130757FxSTAT3+/-) animals after bleomycin exposure. The X-axis shows the different animal genotypes, and the Y-axis shows the fold-change in expression as compared to wild-type. *$P<0.05$ compared with WT and gp130757FxSTAT3+/- animals respectively. Collagen α1 gene expression was measured by Real-Time RT-PCR with mRNA samples isolated from lung tissues, and total collagen synthesis was estimated by hydroxyproline concentrations in lung tissue extracts with high pressure liquid chromatography.

Figure 4. Hyper-activation of gp130 mediated STAT3 pathway increases susceptibility of transgenic animals to bleomycin induced pulmonary fibrosis. The bar graph shows results of quantitative measuring of lung collagen accumulation by high pressure liquid chromatography. Levels of hydroxyproline per 104 lung cells (Y-axis) are significantly elevated in the lung tissue of mice (genotypes shown on X-axis) with hyper-activated STAT3 after bleomycin exposure compared with ΔSTAT3 and WT animals.

Figure 5. IL-6 stimulation dramatically increases collagen alpha-1 gene activation in fibroblasts derived from the lung tissue of mice with hyper-activated STAT3. The bar graph shows collagen α1 promoter activity (Y-axis) in lung fibroblasts derived from wild-type (WT), hyper-activated STAT3 (gp130757F), and STAT3 defective (gp130757FxSTAT3+/-) animals in the presence of gp130 activation ligand – IL-6, at different concentrations, shown on the X-axis. **$P<0.05$ compared with WT and gp130757FxSTAT3+/- fibroblasts.

Figure 6. STAT3C induced expression of collagen in human lung fibroblasts. Spontaneous and TGFβ1-induced expression of collagen 1α1 transcripts in 2 different lines of human lung fibroblasts are shown, non-infected and infected with STAT3C or LacZ lentivirus expressing constructs. Bars are mean ±SE of the triplicates of one qRT-PCR reaction.

Figure 7. Gp130-STAT1/3 signaling promotes fibrosis independent of Smad3. STAT3 activation regulates lung fibrosis and collagen synthesis independent of Smad3. The role of Smad3 signalling in the development bleomycin-induced fibrosis assessed by treating Smad3 null mice ($Sm3^{-/-}$) and gp130757F/757F mice cross-bred with Smad3 null mice (F/F; $Sm3^{-/-}$) with bleomycin. Fibrosis was assessed by Masson's trichrome stain of lung tissue and total collagen accumulation 21 days after bleomycin treatment. $Sm3^{-/-}$ had no signs of fibrosis 21 days after bleomycin treatment, in contrast F/F; $Sm3^{-/-}$ mice showed extensive fibrosis of the lung parenchyma 21 days after bleomycin treatment. Images are representative of n=3 mice and scale bar = 20μm. n=3, *$p<0.05$.

Figure 8. Proliferation is slowed dramatically in cells with constitutively high STAT3 activity. Relative proliferation rates of fibroblasts were determined using the Cell Proliferation Biotrak ELISA System™, version 2 (Amersham Biosciences™, Piscataway, NJ), according to the manufacturer's directions. Cells were seeded in a 96-well plate at a density of 5,000 cells per well. Cells were quiesced in serum-free media for 24 hours prior to stimulation, and BrdU was added at 24 hours post stimulation. The assay was performed at 48 hours post stimulation. * = $p<0.001$.

Figure 9. Inhibitory effect of STA-21 on SOCS3 expression (compared to beta3-integrin). Bar graph shows expression of SOCS3 mRNA in A as compared to beta3-integrin mRNA in B (Y-axis) in normal human lung fibroblasts at baseline or following exposure to Oncostatin™ M (2 μg/ml) in the absence or presence of STA-21 (20 μM) for 48hr. Gene expression was measured using quantitative real time PCR.

Figure 10. STA-21 attenuates the up-regulatory effects of EGF and OSM on OSM-(β)-receptor and IL-6-(α)-receptor expression in human primary lung fibroblasts. Bar graph shows OSMβ-receptor and IL-6α-receptor expression (Y-axis) in normal human lung fibroblasts following exposure to OSM or EGF in the absence or presence of STA-21. STA-21 (20 μM) was added simultaneously with ligands and present through the entire incubation period. Gene expression was measured using quantitative real time PCR.

Figure 11. STA-21 prevents OSM-induced Stat3 expression in human primary bronchial (A) and lung (B) fibroblasts. Bar graph shows expression of STAT3 (Y-axis) in normal human lung fibroblasts at baseline or following exposure to Oncostatin M (2 μg/ml) or EGF (50 ng/ml) in the absence or presence of STA-21 (20 μM) for 48hr. Gene expression was measured using quantitative real time PCR.

Figure 12. STAT3 siRNA validation – primary lung fibroblasts isolated from IPF lung tissue (138-IPF) were transfected with siRNA for STAT3 (STAT3 siRNA was produced by Dharmacon™ and is a "smartpool" of 4 different siRNA's). Two days later total RNA was collected, STAT3 expression evaluated by real-time RT-PCR.

Figure 13. **(A)** shows representative flow cytometry histograms. Thy-1 expression significantly decreased in HLF-STAT3C transduced cells in comparison with non-infected (HLF-Ctrl) and LacZ transduced cells (HLF-LacZ). **(B)** Shows screening of 14 individual lines of human lung fibroblasts isolated from lung tissue samples and anatomical locations for Thy-1 expression and basal levels of Stat3 activation revealed a negative correlation between percentage of Thy-1 positive cells and levels of spontaneous Stat3 posphorylation in the given cell population.

Detailed Description

Any terms not directly defined herein shall be understood to have the meanings commonly associated with them as understood within the art of the invention.

As used herein a 'subject' refers to an animal, such as a bird or a mammal. Specific animals include rat mouse dog cat cow, sheep horse pig or primate. A subject may further be a human, alternatively referred to as a patient. A subject may further be a transgenic animal. A subject may further be a rodent, such as a mouse or a rat.

As used herein, an 'inhibitor' refers to a drug, compound or an agent that restrains or retards a physiological, chemical or activity or function. An inhibitor may cause at least 5% decrease in activity. An inhibitor may also refer to a drug, compound or agent that prevents or reduces the expression, transcription or translation of a gene or protein.

As used herein, 'STAT3' refers to any form of STAT3, including, but not limited to, STAT3α and STAT3β. For example, the human STAT3 nucleotide sequence may correspond to one or more of the sequences in **Table 1** below.

TABLE 1 - Nucleotide Sequences

SEQUENCE	IDENTIFIER

signal transducer and activator of transcription 3 (acute-phase response factor) (STAT3), transcript variant 3, mRNA [Homo sapiens]	NM_213662.1 GI:47458819
signal transducer and activator of transcription 3 (acute-phase response factor) (STAT3), transcript variant 2, mRNA [Homo sapiens]	NM_003150.3 GI:47080105
signal transducer and activator of transcription 3 (acute-phase response factor) (STAT3), transcript variant 1, mRNA [Homo sapiens]	NM_139276.2 GI:47080104
signal transducer and activator of transcription 3 (acute-phase response factor) (STAT3); and signal transducer and activator of transcription 5A (STAT5A), on chromosome 17 [Homo sapiens]	NG_007370.1 GI:166706892
signal transducer and activator of transcription 3 (acute-phase response factor), mRNA (cDNA clone MGC:1607 IMAGE:3347434), complete cds [Homo sapiens]	BC000627.2 GI:33988567
signal transducer and activator of transcription 3 (acute-phase response factor), mRNA (cDNA clone MGC:23173 IMAGE:4909141), complete cds [Homo sapiens]	BC014482.1 GI:15680253
Synthetic construct Homo sapiens clone HAIB:100066468; DKFZo008D0317 signal transducer and activator of transcription 3 (acute-phase response factor) protein (STAT3) gene, encodes complete protein [Homo sapiens]	EU831439.1 GI:190690702
Synthetic construct Homo sapiens clone HAIB:100066560; DKFZo004D0318 signal transducer and activator of transcription 3 (acute-phase response factor) protein (STAT3) gene, encodes complete protein [Homo sapiens]	EU831531.1 GI:190689350

Examples of human STAT3 protein sequence may be found in **Table 2** below.

TABLE 2 – Amino Acid Sequences

SEQUENCE	**IDENTIFIER**
STAT3 [Homo sapiens]	AAK17196.1 GI:13272532
signal transducer and activator of transcription 3 isoform 1 [Homo sapiens]	NP_644805.1 GI:21618340
signal transducer and activator of transcription 3 isoform 2 [Homo sapiens]	NP_003141.2 GI:21618338
signal transducer and activator of transcription 3 isoform 3 [Homo sapiens]	NP_998827.1 GI:47458820
signal transducer and activator of transcription 3 [Homo sapiens]	AAB84254.1 GI:2613014

signal transducer and activator of transcription 3 [Homo sapiens]	P40763.2 GI:48429227

STAT3 INHIBITORS

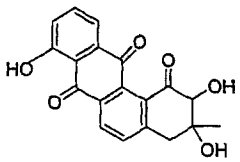
As used herein, a 'STAT3 inhibitor' refers to a compound which is capable of preventing or decreasing the activation of STAT3, or decreasing the activity of STAT3. Activation of STAT3 occurs at the molecular level by phosphorylation and dimerization, which allows for STAT3 to pass through the nuclear membrane and bind DNA. This binding allows STAT3 to act as a transcription factor, and thereby affect gene expression. Accordingly, inhibition of STAT3 by a STAT3 inhibitor may reduce phosphorylation, dimerization, nuclear membrane translocation and/or DNA binding by STAT3. A STAT3 inhibitor may also be capable of reducing the transcription of or translation of STAT3. STAT3 inhibitors may be small molecules, peptides, peptidomimetics, RNA compounds and the like. Examples of STAT 3 inhibitors include, but are not limited to, Lipoxin A4 (LXA4), LY294002, STA-21, Leflunomide, 6-nitro-1-benzothiophene 1,1-dioxide (CID: 2779853), Cucurbitacin D, Tetrahydro- Cucurbitacin I, NSC628869, NSC94743, NSC521777, NSC49451, NSC106399, and NSC135075, or analogues thereof. Other examples of STAT3 inhibitors may include antisense RNA compounds. An example of an antisense RNA compound that is a STAT3 inhibitor is an RNA compound having a nucleotide sequence corresponding to SEQ ID NO: 1. A STAT3 inhibitor may be selected from one or more of: STAT3 Peptidomimetics; Protein Inhibitor of Activated STAT3 (PIAS3); STAT3 Antisense RNA; STAT3 siRNA; Lipoxin A4 (LXA4); LY294002; STA-21; Leflunomide; 6-nitro-1-benzothiophene 1,1-dioxide (CID: 2779853); Cucurbitacin D; Tetrahydro- Cucurbitacin I; NSC628869; NSC94743; NSC49451; NSC521777; NSC106399; and NSC135075; analogues thereof; pharmaceutically acceptable salts thereof; prodrugs thereof; metabolites thereof; and a pharmaceutical composition comprising one or more of the above.

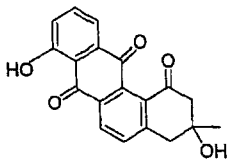
STAT3 inhibitors and methods for using STAT3 inhibitors to modulate activation or expression of STAT3 for the treatment of ILD are described herein. In some embodiments, the ILD is a lung fibrotic disease, for example IPF.

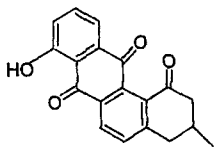
A STAT3 inhibitor, may be administered, in a therapeutically effective amount, to a subject suspected of having an ILD, or previously having had an ILD. A compound having STAT3 inhibitor activity, may be administered, in a therapeutically effective amount, to a subject suspected of having an ILD, or previously having had an ILD.

STAT3 inhibitors that may be useful in some embodiments of the invention may include one or more of: a STAT3 peptidomimetic; Protein Inhibitor of Activated STAT3 (PIAS3); a STAT3 antisense RNA; a STAT3 siRNA; Lipoxin A_4 (LXA_4); LY294002; STA-21; Leflunomide; 6-nitro-1-benzothiophene 1,1-dioxide (CID: 2779853); Cucurbitacin D; Tetrahydro- Cucurbitacin I; NSC628869; NSC94743; NSC49451; NSC521777; NSC106399; and NSC135075; salts thereof; analogues thereof; variants thereof; pharmaceutically acceptable salts thereof; a pro-drugs thereof; a metabolites thereof; and a pharmaceutical composition comprising one or more of the above.

Small molecule inhibitors of STAT3 are described in WO 06/091837, including the use of STA-21 (shown below with two analogues thereof) relating to cancer.

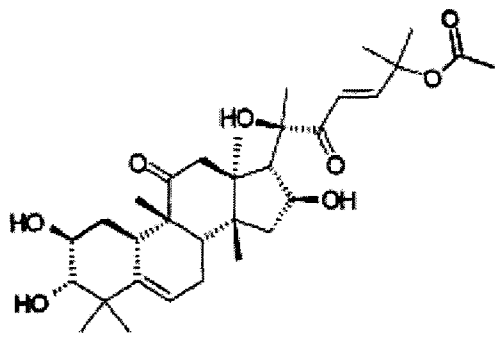




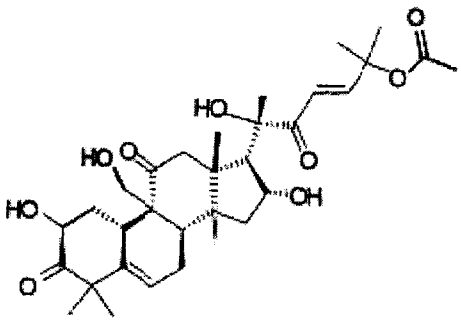


Additionally, methods for treatment of cancer using STAT3 inhibitors (including Cucurbitacin I and related compounds –see below) are described in WO 02/078617.

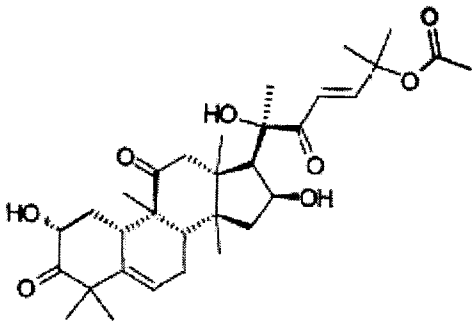
NSC135075 (Cucurbitacin Q)



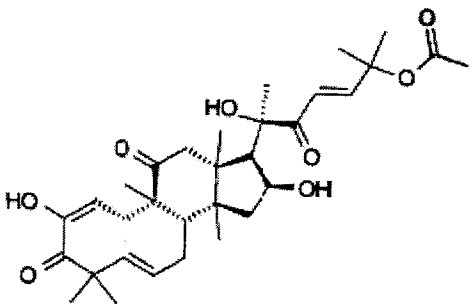
NSC94743 (Cucurbitacin A)



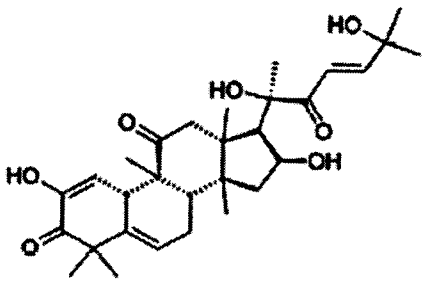
NSC49451 (Cucurbitacin B)



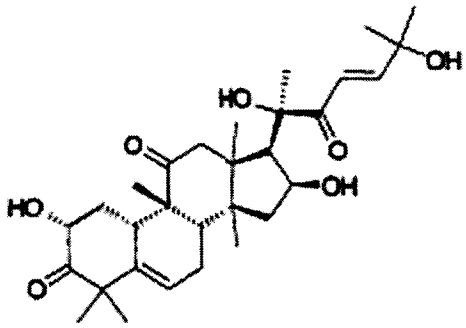
NSC106399 (Cucurbitacin E)



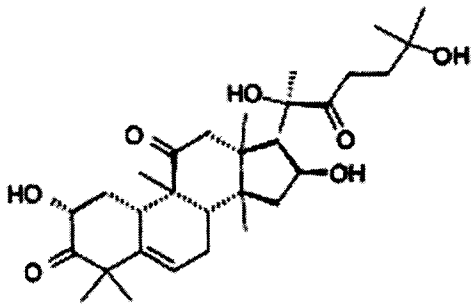
NSC521777 (Cucurbitacin I or JSI-124)



Cucurbitacin D



Tetrahydro- Cucurbitacin I



STAT3 inhibitors may be RNA compounds. The STAT3 inhibitors may be antisense RNA compounds, as described herein. The STAT3 inhibitors may be antisense RNA compounds which are capable of modulating the expression or activity of STAT3. The anti-sense RNA compounds of the invention may have a sequence corresponding to SEQ ID NO: 1 (i.e. 5′-CAGCCTCTCTGCAGAATTCAA-3′). An antisense RNA compound having a sequence corresponding to SEQ ID NO:1 was capable of reducing STAT3 expression in human fibroblasts (data not shown). The RNA compounds of the invention may alternately have a nucleotide sequence determined to be effective for the modulation of STAT3 using methods and techniques known in the art, and/or as described herein.

A STAT3 inhibitor may be used to prevent or inhibit the expression or deposition of collagen from a cell or tissue. A STAT3 inhibitor may be used to prevent or inhibit the expression or deposition of collagen from a fibroblast. A STAT3 inhibitor may be administered to a fibroblast cell or fibroblast-containing tissue in culture to prevent or inhibit the deposition of collagen from a fibroblast. A STAT3 inhibitor may be administered to a fibroblast or fibroblast-containing tissue in a subject, by administering the STAT3 inhibitor to a subject, to prevent or inhibit the deposition of collagen from a fibroblast. A STAT3 inhibitor may be used to prevent or inhibit the differentiation of a fibroblast to a myofibroblast. A STAT3 inhibitor may be administered to a fibroblast cell or fibroblast-containing tissue in culture to prevent or inhibit the differentiation of a fibroblast to a myofibroblast. A STAT3 inhibitor may be administered to a fibroblast or fibroblast-containing tissue in a subject, by administering the STAT3 inhibitor to a

subject, to prevent or inhibit the differentiation of a fibroblast to a myofibroblast. In particular, where a fibroblast is a lung fibroblast and the subject has an ILD. Furthermore, the subject may have a fibrotic lung disease, such as IPF.

The terms 'RNA' or 'RNA compound' as used herein includes RNA molecules, RNA segments and RNA fragments. RNA may be single stranded, double stranded, synthetic, isolated, partially isolated, essentially pure or recombinant. RNA compounds may be naturally occurring, or they may be altered such that they differ from naturally occurring RNA compounds. Alterations may include addition, deletion, substitution or modification of existing nucleotides. Such nucleotides may be either naturally occurring, or non-naturally occurring nucleotides. Alterations may also involve addition of non-nucleotide material, for instance at the end or ends of an existing RNA compound, or at a site that is internal to the RNA compound (i.e. at one or more nucleotides).

In some embodiments, there are provided RNA compounds that are STAT3 inhibitors, and methods of their use. The RNA compounds of the invention are capable of target-specific modulation of gene expression and typically exert their effect either by mediating degradation of the mRNA products of the target gene, or by preventing protein translation from the mRNA of the target gene. Such RNA compounds may thus also be referred to as 'RNA interference compounds'. The overall effect of interference with mRNA function is modulation of expression of the product of a target gene. In the context of this invention, "modulation" means either inhibition or stimulation – i.e. either a decrease or an increase in expression. This modulation can be measured in ways which are routine in the art, for example by Northern blot assay or reverse transcriptase PCR of mRNA expression, Western blot or ELISA assay of protein expression, or by immunoprecipitation assay of protein expression. Effects on cell proliferation, cell viability or tumour growth can also be measured.

In some embodiments, the RNA compounds used to inhibit STA3 may be antisense RNA compounds. Antisense RNA compounds are typically single stranded RNA compounds which bind to complementary RNA compounds, such as target mRNA molecules, and block translation from the complementary RNA compounds by sterically interfering with the normal translational machinery. This process is usually passive, in that it does not require or involve additional enzymes to mediate the RNA interference process. Specific targeting of antisense RNA compounds to inhibit the expression of a desired gene may generally involve designing the antisense RNA compound to have a

homologous, complementary sequence to the desired gene. Complete sequence identity is not necessary for the RNA interference effect. The antisense RNA compounds may include any RNA compound with sufficient complementary homology to STAT3 to bind to the STAT3 mRNA transcript causing a reduction in translation of STAT3 protein.

In other embodiments, the RNA compounds used to inhibit STAT3 may be small interfering RNA (siRNA) compounds. Typically, siRNA compounds are short double stranded RNA compounds between 4 and 49 nucleotides in length. More preferably, siRNA compounds are between 16 to 29 nucleotides in length, even more preferably between 18 to 23 nucleotides in length and most preferably between 21-23 nucleotides in length. The siRNA compounds may include short nucleotide 'overhangs' on each end, which are single stranded extensions which are not paired with a complementary base on the opposite strand. The overhangs would preferably be on the 3' end of each strand of the siRNA compound, and are typically 1-3 nucleotides in length. The siRNA compounds of the present invention may be synthesized as individual strands which are subsequently annealed to produce the double stranded siRNA compound. Alternately, the siRNA compounds may be derived from a short hairpin RNA (siRNA) molecule, or from a longer RNA compound, which has been processed by the cellular enzyme called dicer, which processes the longer RNA compounds to produce siRNA compounds. Generally, siRNA compounds mediate RNA interference via an enzyme-dependent process in which the target mRNA is degraded, such that it can no longer be translated into its associated protein product. The double stranded siRNA compounds may be separated into single stranded molecules and integrated into an activated 'RISC complex'. After integration into the RISC, siRNAs may base-pair to their target mRNA and induce cleavage of the mRNA, thereby preventing it from being used as a translation template.

Design of gene specific antisense RNA compounds, including nucleotide sequence selection and additionally appropriate alterations, are known to persons of skill in the art. Specific targeting of siRNA compounds to modulate expression of a desired gene is generally related to the degree of homology between the siRNA compound and the target gene. Design features to optimize the efficacy and specificity of an antisense RNA compound may depend on the specific sequence chosen for the design of the RNA compound. Numerous examples of methods for designing and optimizing antisense RNA compounds are found in the scientific literature – (for example, Pan and Clawson 2006; Patzel 2007; and Peek and Behlke 2007). There are also many computer based tools for

designing antisense RNA compounds, which may, for instance, use algorithms or other rule-based formulae to determine optimal antisense RNA compounds. It would thus be within the abilities of a person of skill in the art to design a large number of different antisense RNA compounds, which would be expected to inhibit a target gene. Exact sequence identity is not necessary for the siRNA compound to modulate expression of the target gene. The antisense RNA compounds may include any RNA compounds which have sequence homology to the STAT3 gene and which are capable of modulating the expression of STAT3 protein. The description provides non-limiting examples of RNA compounds which modulate the expression of STAT3 and are thus STAT3 inhibitors. Other examples of RNA compounds which are capable of modulating expression of STAT3 are found in: US 6,159,694; US 6,727,064; US 7,098,192; and US 7,307,069. Examples of antisense compounds and antisense target sequences that inhibit the expression of human STAT3 may be found in **Table 3** below.

TABLE 3

Source	**Sequences**
US 6,159,694	Targeted to 8 to 30 bases selected from the following cgagggaacaagccccaacc (SEQ ID NO:2) OR ggcac cccggcttgg cgctgtctct ccccctcggc tcggagaggc ccttcggcct gagggagcct cgccgcccgt ccccggcaca cgcgcagccc cggcctctcg gcctctgccg gagaaacagg atggcccaat ggaatcagct acagcagctt gacacacggt acctggagca gctccatcag ctctacagtg acagcttccc aatggagctg cggcagtttc tggccccttg gattgagagt caagattggg catatgcggc cagcaaagaa tcacatgcca ctttggtgtt tcataatctc ctgggagaga ttgaccagca gtatagccgc ttcctgcaag agtcgaatgt tctctatcag cacaatctac gaagaatcaa gcagtttctt cagagcaggt atcttgagaa gccaatggag attgcccgga ttgtggcccg gtgcctgtgg gaagaatcac gccttctaca gactgcagcc actgcggccc agcaaggggg ccaggccaac caccccacag cagccgtggt gacggagaag cagcagatgc tggagcagca ccttcaggat gtccggaaga gagtgcagga tctagaacag aaaatgaaag tggtagagaa tctccaggat gactttgatt tcaactataa aaccctcaag agtcaaggag acatgcaaga tctgaatgga aacaaccagt cagtgaccag gcagaagatg cagcagctgg aacagatgct cactgcgctg gaccagatgc ggagaagcat cgtgagtgag ctggcggggc ttttgtcagc gatggagtac gtgcagaaaa ctctcacgga cgaggagctg gctgactgga agaggcggca acagattgcc tgcattggag gcccgcccaa catctgccta gatcggctag aaaactggat aacgtcatta gcagaatctc aacttcagac ccgtcaacaa attaagaaac tggaggagtt gcaccaaaaa gtttcctaca aaggggaccc cattgtacag caccggccga tgctggagga gaggatcgtg gagctgttca gaaacttaat gaaaagtgcc tttgtggtgg agcggcagcc ctgcatgccc

atgcatcctg accggcccct cgtcatcaag accggcgtcc
agttcactac taaagtcagg ttgctggtca agttccctga
gttgaattat cagcttaaaa ttaaagtgtg cattgacaaa
gactctgggg acgttgcagc tctcagagga tcccggaaat
ttaacattct gggcacaaac acaaaagtga tgaacatgga
agaatccaac aacggcagcc tctctgcaga attcaaacac
ttgaccctga gggagcagag atgtgggaat gggggccgag
ccaattgtga tgcttccctg attgtgactg aggagctgca
cctgatcacc tttgagaccg aggtgtatca ccaaggtctc
aagattgacc tagagaccca ctccttgtca gttgtggtga
tctccaacat ctgtcagatg ccaaatgcct gggcgtccat
cctgtggtac aacatgctga ccaacaatcc caagaatgtg
aacttcttca ctaagccgcc aattggaacc tgggaccaag
tggccgaggt gctcagctgg cagttctcgt ccaccaccaa
gcgggggctg agcatcgagc agctgacaac gctggctgag
aagctcctag ggcctggtgt gaactactca gggtgtcaga
tcacatgggc taacttctgc aaagaaaaca tggctggcaa
gggcttctcc tactgggtct ggctagacaa tatcatcgac
cttgtgaaaa agtatatctt ggccctttgg aatgaagggt
acatcatggg tttcatcagc aaggagcggg agcgggccat
cttgagcact aagcccccag gcaccttcct gctgcgcttc
agtgaaagca gcaaagaagg aggcgtcact ttcacttggg
tggagaagga catcagcggt aagacccaga tccagtccgt
ggaaccatac acaaagcagc agctgaacaa catgtcattt
gctgaaatca tcatgggcta taagatcatg gatgctacca
atatcctgtt gtctccactt gtctatctct atcctgacat
tcccaaggag gaggcattcg ggaagtattg tcggccagag
agccaggagc atcctgaagc tgacccaggt agcgctgc
(SEQ ID NO:3) OR
cagcaataccattgacctgc (SEQ ID NO:4) OR
t tcggggcggc ggcgcagact gggaggggga
gccgggggtt ccgacgtcgc agccgaggga acaagcccca
accggatcct ggacaggcac cccggcttgg cgctgtctct
ccccctcggc tcggagaggc ccttcggcc (SEQ ID NO:5)OR
gcccaat ggaatcagct acagcagctt gacacacggt
acctggagca gctccatcag ctctacagtg acagcttccc
aatggagctg cggcagtttc tggccccttg gattgagagt
caagattggg catatgcggc cagcaaagaa tcacatgcca
ctttggtgtt tcataatctc ctgggagaga ttgaccagca
gtatagccgc ttcctgcaag agtcgaatgt tctctatcag
cacaatctac gaagaatcaa gcagtttctt cagagcaggt
atcttgagaa gccaatggag attgcccgga ttgtggcccg
gtgcctgtgg gaagaatcac gccttctaca gactgcagcc
actgcggccc agcaaggggg ccaggccaac caccccacag
cagccgtggt gacggagaag cagcagatgc tggagcagca
ccttcaggat gtccggaaga gagtgcagga tctagaacag
aaaatgaaag tggtagagaa tctccaggat gactttgatt
tcaactataa aaccctcaag agtcaaggag acatgcaaga
tctgaatgga aacaaccagt cagtgaccag gcagaagatg
cagcagctgg aacagatgct cactgcgctg gaccagatgc
ggagaagcat cgtgagtgag ctggcggggc ttttgtcagc
gatggagtac gtgcagaaaa ctctcacgga cgaggagctg
gctgactgga agaggcggca acagattgcc tgcattggag
gcccgcccaa catctgccta gatcggctag aaaactggat
aacgtcatta gcagaatctc aacttcagac ccgtcaacaa

	attaagaaac tggaggagtt gcaccaaaaa gtttcctaca aaggggaccc cattgtacag caccggccga tgctggagga gaggatcgtg gagctgttca gaaacttaat gaaaagtgcc tttgtggtgg agcggcagcc ctgcatgccc atgcatcctg accggcccct cgtcatcaag accggcgtcc agttcactac taaagtcagg ttgctggtca agttccctga gttgaattat cagcttaaaa ttaaagtgtg cattgacaaa gactctgggg acgttgcagc tctcagagga tcccggaaat ttaacattct gggcacaaac acaaaagtga tgaacatgga agaatccaac aacggcagcc tctctgcaga attcaaacac ttgaccctga gggagcagag atgtgggaat gggggccgag ccaattgtga tgcttccctg attgtgactg aggagctgca cctgatcacc tttgagaccg aggtgtatca ccaaggtctc aagattgacc tagagaccca ctccttgtca gttgtggtga tctccaacat ctgtcagatg ccaaatgcct gggcgtccat cctgtggtac aacatgctga ccaacaatcc caagaatgtg aacttcttca ctaagccgcc aattggaacc tgggaccaag tggccgaggt gctcagctgg cagttctcgt ccaccaccaa gcgggggctg agcatcgagc agctgacaac gctggctgag aagctcctag ggcctggtgt gaactactca gggtgtcaga tcacatgggc taacttctgc aaagaaaaca tggctggcaa gggcttctcc tactgggtct ggctagacaa tatcatcgac cttgtgaaaa agtatatctt ggccctttgg aatgaagggt acatcatggg tttcatcagc aaggagcggg agcgggccat cttgagcact aagcccccag gcaccttcct gctgcgcttc agtgaaagca gcaaagaagg aggcgtcact ttcacttggg tggagaagga catcagcggt aagacccaga tccagtccgt ggaaccatac acaaagcagc agctgaacaa catgtcattt gctgaaatca tcatgggcta taagatcatg gatgctacca atatcctgtt gtctccactt gtctatctct atcctgacat tcccaaggag gaggcattcg ggaagtattg tcggccagag agccaggagc atcctgaagc tgacccaggt agcgctgccc catacctgaa gaccaagttt atctgtgtga caccaacgac ctgcagcaat accattgacc tgccgatgtc cccccgcgct ttagattcat tgatgcagtt tggaaataat ggtgaaggtg ctgaaccctc agcaggaggg cagtttgagt ccctcacctt tgacatggag ttg (SEQ ID NO:6) OR cccatgtgaggagctgagaacggaagct (SEQ ID NO:7) OR ga ggcgcctacc tgcattctgc cacccctcac acagccaaac cccagatcat ctgaaactac taactttgtg gttccagatt ttttttaatc tcctacttct gctatctttg agcaatctgg gcacttttaa aaatagagaa atgagtgaat gtgggtgatc tgcttttatc taaatgcaaa taaggatgtg ttctctgaga cccatgatc (SEQ ID NO:8)
US 6,727,064	Targeted to gaagcagcagatgctggagc (SEQ ID NO:9) AND caacatgctgaccaacaatcccaagaatg (SEQ ID NO:10)
US 7,098,192	Consisting of gactcttgcaggaagcggct (SEQ ID NO:11)
US 7,307,069	Consisting of ttggcttctcaagatacctg (SEQ ID NO:12)

ADMINISTRATION

STAT3 inhibitors and conjugates of STAT3 inhibitors with targeting moieties and/or cell uptake moieties may be administered in any of a variety of known ways. Examples of methods that may be suitable for the administration of a compound include orally, intravenous, inhalation, intramuscular, subcutaneous, topical, intraperitoneal, intra-rectal or intra-vaginal suppository, sublingual, and the like. A composition comprising a STAT3 inhibitor may be administered as a sterile aqueous solution, or may be administered in a fat-soluble excipient, or in another solution, suspension, patch, tablet or paste format as is appropriate. A composition comprising a STAT3 inhibitor may be formulated for administration by inhalation. For instance, a composition comprising a STAT3 inhibitor may be combined with an excipient to allow dispersion in an aerosol. Examples of inhalation formulations will be known to those skilled in the art or are described herein. Other agents may be included in combination with the STAT3 inhibitor to aid uptake or metabolism, or delay dispersion within the host, such as in a controlled-release formulation. Examples of controlled release formulations will be known to those of skill in the art, and may include microencapsulation, embolism within a carbohydrate or polymer matrix, etc. Other methods known in the art for making formulations are found in, for example, "Remington's Pharmaceutical Sciences", (19^{th} edition), ed. A. Gennaro, 1995, Mack Publishing Company, Easton, Pa.

The dosage of the STAT3 inhibitors may vary depending on the route of administration (oral, intravenous, inhalation, etc.) and the form in which the STAT3 inhibitor is administered (solution, controlled release etc.). Determination of appropriate dosages is within the ability of one of skill in the art. As used herein, an 'effective amount', a 'therapeutically effective amount', or a 'pharmacologically effective amount' of a medicament refers to an amount of a medicament present in such a concentration to result in a therapeutic level of drug delivered over the term that the drug is used. This may be dependent on mode of delivery, time period of the dosage, age, weight, general health, sex and diet of the subject receiving the medicament. Methods of determining effective amounts are known in the art.

Systemic delivery of a STAT3 inhibitor to multiple sites in the body could potentially have detrimental side effects. STAT3 is a transcription factor that has numerous targets in different tissue and cell types, and as such, it could be potentially beneficial to restrict delivery of a STAT3 inhibitor to the target tissue or cell in which

inhibition of STAT3 is desired. A STAT3 inhibitor may be selectively delivered to the target tissue or cell in which inhibition of STAT3 is desired. For example, a disease of the lung, such as idiopathic pulmonary fibrosis, may be characterized by excessive fibrosis in lung tissue. It is contemplated that the selective delivery of a STAT3 inhibitor to lung tissue may inhibit or reduce fibrosis in the lung, without producing significant side effects in other tissues of the body. Methods are provided herein for selective delivery to the lung tissue that may include, but are not limited to, the use of aerosols, nebulizers, dry powder inhalers, metered dose inhalers, nanoparticles, or conjugation to compounds which enhance selective delivery to the lung, for instance dextran, porous particles, lipidic compounds, or liposomal compounds. Furthermore, targeting moieties are described below, which may assist in directing STAT3 inhibitors to fibrotic lung cells. Some examples of devices to aid such delivery could include a dry powder inhaler (for example, US 5,875,776), a continuous flow nebuliser (for example US 5,277,175) or a metered dose inhaler (for example, US 6,325,062). Other examples of compositions or methods to facilitate localized tissue delivery would be apparent to one of skill in the art. It can also be appreciated that, in addition to restricting delivery of a STAT3 inhibitor to specific tissues or cell types in the body of a subject, partial inhibition of STAT3 in a tissue or cell could be an advantageous method of administration. Partial inhibition of STAT3, as opposed to complete or total inhibition, may provide therapeutic effects in the target tissue, with a reduction in the potential side effects that might be seen with complete or total inhibition of STAT3. Examples of methods that could be used for partial inhibition of STAT3 may include modulation of dosage or use of excipients or formulations that allow slow release of the STAT3 inhibitor. Other examples of methods that could be used for partial inhibition of STAT3 would be known to one of skill in the art.

A method of treating fibrotic lung tissue by administering, to a subject, an effective amount of a compound or composition which inhibits STAT3 is provided herein. Administration by inhalation would be a preferable route of administration for fibrotic lung tissue. Alternatively, systemic administration, for example by intravenous injection or infusion, may also be useful. Particularly, where the STAT3 inhibitor may be administered in an inactivated form and activated only at the site of fibrotic disease. Furthermore, the STAT3 inhibitor may be coupled to a targeting moiety to deliver a STAT3 inhibitor to slow growing, differentiated, hypertrophic fibroblasts in fibrotic lung tissue. Methods are known in the art for formulating antibodies together with therapeutic

compositions for the inhalation (for example, 6,485,707, 6,518,239, 6,544,497, and 6,551,578. Furthermore, STAT3 inhibitors may be coupled to a targeting moiety and/or a cell uptake moiety to form a conjugate as described herein. A targeting moiety may be, for example, an antibody having specificity for: fibronectin (FN1); collagen 1α1 (COL1A1); connective tissue growth factor (CTFG); transforming growth factor beta 1 (TGFB1); α-smooth muscle actin (α-SMA); or interleukin-6 (IL-6). For example, AP6107 is a COL1A1 antibody (C-term) from ABGENT™ or ab7817, which is a monoclonal antibody [1A4] to alpha smooth muscle actin antibody (ab7817) from abcam™. Antibodies to be used as a targeting moiety as described herein are preferably suitable for therapeutic use and may be humanized MAbs.

A person of skill may determine for each individual case the best treatment strategy, with respect to route of administration, active substance or composition to be administered and dose and treatment protocol.

A "targeting moiety" as used herein, is an entity that when coupled, either directly or indirectly, to a STAT3 inhibitor or alternatively associated with a STAT3 inhibitor (herein referred to as a conjugate), facilitates delivery of the STAT3 inhibitor to a target cell. The delivery facilitated by the targeting moiety does not require the exclusion of non-target cells, but the preferential delivery to target over non-target cells. In some embodiments the conjugate of the STAT3 inhibitor and targeting moiety would retain specific binding to the target cells and STAT3 inhibitory activity. In alternative embodiments, the conjugate may be coupled in such a manner that the targeting moiety would retain specific binding, but may not have STAT3 inhibitory activity until after binding to the target cell and/or translocation of the target cell membrane. The target cells may be cells that are associated with an ILD. A target cell may be a fibrotic lung cell. A target cell may be a slow growing, differentiated, hypertrophic fibroblast cell in fibrotic lung tissue. A targeting moiety, as described herein, may be selected from, but not limited to, the group consisting of: antibodies; nucleic acids; receptor directed ligands; long-circulating particles; etc. The antibodies may be monoclonal antibodies or human or humanized monoclonal antibodies or fragments thereof as described herein. The receptor directed ligands may be a ligand having binding specificity for a receptor associated with said target cell or a ligand fragment having binding specificity for a receptor associated with said target cells. The long-circulating particles, may be PEG-coated liposomes capable of localizing at a ILD site by extravasation, and containing encapsulated or

surface bound STAT3 inhibitor. A conjugate may further comprise a cell uptake moiety as described herein. Alternatively, the targeting moiety may itself have cell uptake activity in relation to the target cell. Furthermore, a carrier molecule, such as HSA or a polymer, which can be loaded with STAT3 inhibitors and coupled to a targeting moiety. Wherein the STAT3 inhibitor is a peptide or protein, an antibody-peptide (STAT3 inhibitor) conjugate may be used. Such conjugates are known in the art (for example U.S. Pat. No. 5,650,150). Methods for linking the STAT3 inhibitor to a targeting moiety or cell uptake moiety may include one or more of the following: covalent bonding; non-covalent bonding; chemical cross-linking; heterobifunctional cross-linking (such as hydroxyl, amino, amido, or sulfhydryl reactive groups in the STAT3 inhibitor and in the targeting moiety). Such bonds may be, for example, peptide bonds, disulfide bonds, thioester bonds, amide bonds, thioether bonds, etc. For Example, conjugates of monoclonal antibodies with drugs have been described in Morgan and Foon (1984).

A "conjugate", as used herein, is a STAT3 inhibitor coupled, either directly or indirectly, to at least one targeting moiety or associated with at least one targeting moiety. Alternatively a conjugate may be further bound, directly or indirectly, to at least one cell uptake moiety. Alternatively, the targeting moiety may itself have cell uptake activity in relation to the target cell. In some embodiments the conjugate of the STAT3 inhibitor and targeting moiety would retain the capability to bind specifically to the target cells and retain STAT3 inhibitory activity. In alternative embodiments, the conjugate may be coupled in such a manner that the targeting moiety would retain specific binding, but may not have STAT3 inhibitory activity until after binding to the target cell and/or translocation of the target cell membrane.

A "cell uptake moiety" as described herein, may be any entity that improves cellular uptake of the conjugate as a whole or of the STAT3 inhibitor. Conjugates may be, for example, fibrotic cell surface receptor ligands or other translocation entities.

STAT3 AND INTERSTITIAL LUNG DISEASE (ILD)

Compounds are described herein that are capable of interfering with molecular events leading to the activation of STAT3 in relation to ILD. Current primary treatment regimens for fibrotic diseases such as IPF include glucocorticoids, for example prednisone. Other agents have been used with and without glucocorticoids, including cyclophosphamide, azathioprine. methotrexate, colchicine, penicillamine, and

cyclosporine. There is no direct evidence that such treatments improve the survival of subjects with IPF, and the treatments do not appear to effectively reduce or reverse fibrosis.

The present application demonstrates a significant difference in the level of activated STAT3 (pSTAT3) between fast growing IPF fibroblasts, slow growing IPF fibroblasts, and normal fibroblasts after stimulation of the fibroblasts with the cytokine interleukin-6 (IL-6). A correlation between activated STAT3 levels and fibroblast morphology is also shown. In addition, a significant correlation between levels of activated STAT3 and collagen expression in the lung and collagen α1 promoter activity in lung fibroblasts is also shown. These results suggest that STAT3 is involved in the differentiation of fibroblasts/myofibroblasts, and in the development and progression of fibrosis in IPF. Furthermore, STA-21 and an siRNA both show inhibition of STAT3.

As used herein, 'fibrosis' refers to the formation of excessive or abnormal fibrous tissue, generally as a reparative or reactive process. Although most connective tissue has fibrillar elements, fibrous tissue is generally laid down at a wound site well vascularised at first (granulation tissue), but later avascular and dominated by collagen rich extracellular matrix. Fibrosis is often associated with increased expression and deposition of connective tissue elements, for example collagen. Fibrosis may also be associated with elevated levels of fibroblasts and/or myofibroblasts.

As used herein, an 'Interstitial Lung Disease' (ILD) refers to a large number of conditions that involve the parenchyma of the lung—the alveoli, the alveolar epithelium, the capillary endothelium, and the spaces between these structures, as well as the perivascular and lymphatic tissues. This heterogeneous group of disorders is classified together because of similar clinical, roentgenographic, physiologic, or pathologic manifestations. These disorders are often associated with considerable morbidity and mortality, and there is little consensus regarding the best management of most of them. The ILDs can be classified into two groups based on the major underlying histopathology: (1) those associated with predominant inflammation and fibrosis, and (2) those with a predominantly granulomatous reaction in interstitial or vascular areas. Each of these groups can be further subdivided according to whether the cause is known or unknown. For each ILD there may be an acute phase, and there is usually a chronic one as well. Rarely, some are recurrent, with intervals of subclinical disease. Sarcoidosis, idiopathic pulmonary fibrosis (IPF), and pulmonary fibrosis associated with connective tissue

disorders are the most common ILDs of unknown etiology (Harrison's Principles of Internal Medicine, 15th edition. Braunwald E. *et al.* editors. McGraw-Hill, New York; or in The Merck Veterinary Manual, 9th edition. Kahn, CM *et al*, editors. Merck & Co., Inc. Whitehouse Station New Jersey).

As used herein, a 'fibrotic lung disease' refers a fibrotic disease that affects the lung tissue. Examples of lung diseases which are often associated with fibrosis, and may thus be considered fibrotic lung diseases, may include idiopathic pulmonary fibrosis, usual interstitial pneumonia (UIP), nonspecific interstitial pneumonia (NSIP), bronchiolitis obliterans organizing pneumonia (BOOP), respiratory bronchiolitis-associated interstitial lung disease (RBILD), desquamative interstitial pneumonia (DIP), radiation pneumonitis; and acute interstitial pneumonia (AIP), lymphoid interstitial pneumonia (LIP), and Hamman-Rich syndrome. A UIP pattern can be seen in patients with other conditions such as asbestosis, connective tissue diseases, chronic hypersensitivity pneumonitis and certain drug-induced lung diseases. Fibrotic lung diseases, as used herein may include, idiopathic pulmonary fibrosis (IPF), pulmonary fibrosis, acute lung injury, hypersensitivity pneumonitis, and drug-induced lung fibrosis.

As used herein, 'hypertrophic' refers to an enlarged or overgrown morphology. At the cellular level, hypertrophy is often associated with a differentiation of the cell to alter its function, in addition to its size and shape. This differentiation may be associated with altered expression of different genes and/or proteins in the cell.

As used herein, a 'fibroblast' refers to a cell ubiquitous in connective tissue from which connective tissue develops. Fibroblasts make, in addition to other compounds, collagens, glycosaminoglycans, reticular and elastic fibers, and glycoproteins found in the extracellular matrix. Fibroblasts can differentiate into myofibroblasts. Modulation of a fibroblast to a myofibroblast begins with the appearance of a protomyofibroblast. Myofibroblasts exhibit contractile properties and express contractile fibers such as α-smooth muscle actin. Myofibroblasts also express higher levels of connective tissue proteins such as collagen (Desmouliere *et al.* 2005).

ANTIBODY PRODUCTION

STAT3 specific peptides or proteins may be used to produce antibodies by a variety of known methods. Such antibodies may be polyclonal, monoclonal, or may be fragments of antibodies. Alternatively, antigens associated with ILDs, referred to herein

as either target antigens, target proteins, or target peptides, but also collectively known as target antigens, could also be used to generate antibodies specific to cells associated with ILD. For example, target antigens, may be selected from one or more of the following: fibronectin (FN1); collagen 1α1 (COL1A1); connective tissue growth factor (CTFG); transforming growth factor beta 1 (TGFB1); α-smooth muscle actin (α-SMA); or interleukin-6 (IL-6).

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others, may be immunized by injection with a target antigen, which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active X substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

Target antigens used to induce antibodies, generally have an amino acid sequence consisting of at least five amino acids, and more preferably at least 10 amino acids. It may also be preferable that they are identical to a portion of the amino acid sequence of the natural protein, and they may contain the entire amino acid sequence of the target antigen. Short stretches of target antigen amino acids may be fused with those of another protein such as keyhole limpet hemocyanin and antibody produced against the chimeric molecule.

Peptides corresponding to a target antigen sequence may be synthesized using methods known in the art, including the recombinant techniques disclosed in the examples below. Such peptides may also be made to incorporate an N-terminal cysteine to facilitate conjugation to other molecules (e.g. to enhance immunogenicity) with such conjugation being mediated by an agent such as m-maleimidobenzoyl-N-hydroxy-succinimide ester (MBS). Antibodies that specifically react with the peptide may be purified from the antisera by affinity chromatography, for example by using Cellulofine™ (Seikagaku Corporation) conjugated with the peptide. The resulting antibodies may be tested by immunoblotting.

Monoclonal antibodies to target antigens or anti-idiotypic monoclonal antibodies may be prepared using any technique, which provide for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma

technique (Kohler G. *et al.* (1975); Kozbor D. *et al.* (1985); Cote RJ. *et al.* (1983); Cole SP. *et al.* (1984)).

One process for obtaining the hybridomas as described herein involves starting from spleen cells of an animal, e.g. mouse or rat, previously immunized in vivo or from spleen cells of such animals previously immunized in vitro with an antigen and fusing the immunized cells with myeloma cells under hybridoma-forming conditions; and selecting those hybridomas which secrete the monoclonal antibodies which are capable of specifically recognizing the target antigen.

Selected hybridomas are cultured in appropriate culture medium; and then the secreted monoclonal antibodies are recovered; or alternatively the selected hybridoma is implanted into the peritoneum of a mouse and, when ascites has been produced in the animal; the monoclonal antibodies formed from the ascites are recovered. Monoclonal antibodies as described herein may be prepared by conventional in vitro techniques such as the culturing of immobilized cells using e.g. hollow fibers or microcapsules or such as the culturing of cells in homogeneous suspension using e.g. airlift reactors or stirred bioreactors.

In addition, techniques developed for the production of "chimeric antibodies", the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can be used (Morrison SL. *et al.* (1984); Neuberger MS. *et al.* (1984); Takeda S. *et al.* (1985)). Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce target antigen-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries (Burton DR. (1991)). Such single chain antibodies may also be used for production of anti-idiotypic antibodies for use as described herein.

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi R. *et al.* (1989); Winter, G. *et al.* (1991)).

Antibody fragments which contain specific binding sites specific for target antigen or for anti-target antigen antibodies may also be generated. For example, such fragments include, but are not limited to, the F(ab')2 fragments which can be produced by pepsin

digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse WD. *et al.* (1989)). Such fragments when specific for anti-target antigen antibodies may be used for production of anti-idiotypic antibodies or fragments thereof.

Monoclonal antibodies as described herein may be "chimeric", an example of which is an animal antigen-binding variable domain coupled to a human constant domain (Cabilly *et al.*, U.S. Pat. No. 4,816,567; Morrison, S. L. *et al.* (1984); Boulianne, G. L. *et al.* (1984); Neuberger, M. S. *et al.*(1985)). The term "chimeric" antibody describes a polypeptide comprising at least the antigen binding portion of an antibody molecule linked to at least part of another protein such as an immunoglobulin constant domain. However, antibodies as described herein may be conjugated to a variety of moieties including labeling moieties.

Various immunoassays may be used for screening to identify antibodies having a desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between a target antigen and its specific antibody. Monoclonal-based immunoassays utilizing monoclonal antibodies reactive to at least two non-interfering epitopes are preferred, but competitive binding assays may also be employed (Maddox, D. E. *et al.* (1983)).

THERAPEUTIC HUMAN ANTIBODIES

The production of composite human antibodies is known in the art (for example, see published US patent application 20080206239). A therapeutic antibodies may be made comprising multiple segments of human variable region sequence from different human antibodies. Combinations of sequence segments may be selected to avoid T cell epitopes, which can reduce the risk associated with therapeutic antibodies. Furthermore, antibodies may be "humanized". Composite antibodies may be produced from reference monoclonal antibodies (MAb) with a wide range of specificities in which the sequence of the final antibody can comprise a composite of many sequence segments, all of which are

human in origin. It is important to maintain the binding properties of the reference MAb and to filter out potential T cell epitopes (reducing the risk of immunogenicity).

Generally the production of a composite human(ized) antibody is as follows:

1) Variable regions of a reference non-human antibody (if available) may be modeled to determine antigen binding regions;

2) Sequence segments may be sourced from a database of unrelated human antibody variable regions;

3) Composite human variable regions with similar binding properties to the reference antibody may be created; and

4) sequence segments may be screened for the presence of potential T cell epitopes;

5) sequence segments containing T cell epitopes may be removed and composite variable regions (minus T cell epitopes) may then be constructed and tested for antibody binding and selection of lead candidates.

Composite human antibodies may be suitable for therapeutic and *in vivo* diagnostic use with a low potential risk of immunogenicity in patients. Furthermore, the same approach may be used to make Fabs, single chain Fv's and antibody-protein fusions.

Methods are described herein for identifying or screening for compounds that inhibit expression or deposition of collagen from fibroblasts. Such methods may include, but are not limited to administering a compound or compounds to cultured fibroblasts and assaying for activity of the collagen α1 promoter, expression of the collagen gene or production and/or deposition of collagen. As an example, one may analyze the effect of a compound on collagen α1 promoter function by transfecting cells with a plasmid construct containing the collagen α1 promoter fused to the cDNA encoding the luciferase gene cloned from the firefly, as described in, for example, (Wang *et al.* 2005). Other examples of reporter systems suitable for the use in an assay for collagen gene expression would be known to one of skill in the art.

METHODS AND REAGENTS

Human Cell Culture

Human lung fibroblasts were obtained from patients with IPF (IPF-Fb) and from normal control subjects (normal-Fb). Lung tissue biopsies were cut into 1-mm fragments and placed approximately 10 mm apart on the surface of culture dishes with Dulbecco's

modified Eagle's medium supplemented with 10% newborn calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml), and 2.5 μg/ml amphotericin B. Fibroblasts were observed growing out of the tissue fragments after 6 to 8 days, developing into a near confluent monolayer of cells after 3 to 4 weeks. Fibroblast cell lines were characterized immunohistochemically to confirm their purity. Staining with antibodies to cytokeratin, von Willebrand factor, and desmin was negative, indicating that the cultures did not contain significant numbers of epithelial, mesothelial, endothelial, or smooth muscle cells. Greater than 95% of the cells stained positively for vimentin, and between 20 and 30% of the cells were also positive for α-smooth muscle actin, confirming the fibroblast/myofibroblast phenotype of the cell lines. Experiments were conducted on cells between passages 3 and 17. Cells were cultured in Kaighn's F-12K media (Gibco™) supplemented with 10% newborn calf serum (Gibco™) and 1% penicillin/streptomycin solution (Gibco™). Cells were serum-starved for 24 hours prior to experimental stimulation.

Western blot and densitometric analysis of STAT3 activation

IPF and normal fibroblasts were stimulated with 100ng/mL IL-6 for durations ranging from 10 minutes to six hours. Total cell protein was extracted, quantified by DC protein assay, resolved by SDS-PAGE, and transferred to nitrocellulose membrane. Membranes were blocked in Odyssey blocking buffer (LI-COR Biosciences™) and subsequently incubated with mouse anti-STAT3 antibody diluted 1:2500 in Odyssey™ blocking buffer, and rabbit anti-Phospho-STAT3 antibody diluted 1:1000 in Odyssey™ blocking buffer.

Following washes, membranes were incubated with goat-anti-mouse IRDye800™ and goat-anti-rabbit IRDye700 conjugated secondary antibodies at a dilution of 1:3000. Membranes were washed again and then scanned on the Odyssey™ Infrared Imaging System (LI-COR Biosciences™).

Densitometric analysis was performed using Odyssey™ Software version 1.1. A mouse anti-β-tubulin antibody was included in the blot analysis as a protein loading control.

Generation of constitutively active STAT3 expressing fibroblasts

The constitutively active STAT3 plasmid construct (STAT3C) [previously described (Bromberg *et al.* 1999)] was obtained from JE Darnell Laboratory (Rockefeller University, NY). The STAT3C and control lentiviruses were created using the ViraPower Lentiviral Expression System (Invitrogen™), and the pLenti6/V5 Directional TOPO Cloning Kit according to the manufacturer's protocols. Briefly, the STAT3C cDNA was PCR amplified and ligated with the pLenti6/V5 TOPO cloning vector (manufacturer's protocol). Products of the ligation reaction (pLenti6/V5-STAT3C) were transformed into One Shot Stbl3™ chemically competent cells and positive transformants were confirmed by restriction digest. The pLenti6/V5-STAT3C vector was then co-transfected into 293FT cells with the ViraPower™ packaging vectors containing the required viral structural and replicative elements. Virus-containing supernatants were collected 72 hours post-transfection and stored at -80 °C until use. Viral titer was determined and human fibroblasts were transduced with STAT3C lentivirus at an MOI of 5. At two days post-transduction, the selective antibiotic Blasticidin (Invitrogen™) was added to the transduced cells in order to select for cells stably expressing the pLenti6/V5-STAT3C vector. STAT3C is often used to transform non-IPF cells to render them very IPF-like.

As a control for viral transduction, a second lentivirus containing the *lacZ* gene, encoding the β-galactosidase protein, was produced and used for transduction as described above by using the manufacturer-supplied (Invitrogen™) pLenti6/V5-GW/*lacZ* vector in place of the pLenti6/V5-STAT3C vector.

Animals

The $gp130^{757F}$ mice were generated as previously described (Tebbutt *et al.* 2002). The $gp130^{\Delta STAT}$ mice were generated as previously described (Ernst *et al.* 2001). The $gp130^{757F}$/ $gp130^{757F}$:STAT3+/- mice were generated as previously described (Judd *et al.* 2006). Wild-type and mutant mice were littermates and were used at the ages of 8-16 weeks. Animals were anaesthetised with methoxyfluorane and bleomycin (0.05U/kg; Blenoxane) was delivered passively across the nostril.

Cell isolation and culture

Lung fibroblasts were isolated by serial trypsin-collagenase digestion of whole lung. Mouse lungs were excised from the heart and trachea and digested in 0.25% trypsin-

EDTA at 37°C for 30 minutes. Lungs were removed from digest, dissected from remaining connective tissue and finely minced with a scalpel. Tissue was then digested in 1mg/ml collagenase 1 for 1 hour at 37°C. Remaining tissue was removed and a cell pellet was formed by centrifugation of digest. Cells were cultured in 10% serum supplemented DMEM with L-glutamine and actinomycin.

Collagen Production- Mouse lungs

100-mg aliquots of powdered lung tissue (obtained by crushing the tissue while frozen at -70°C) were hydrolyzed in 2 ml of 6 M HCl at 110°C for 16 h. Hydrolysates were mixed with 30 mg activated charcoal and filtered (Millipore™, type DA, pore size 0.65 μm). A 100-μl aliquot of a 1-in-10 dilution of filtered hydrolysate was dried using a centrifugal vacuum concentrator. Hydroxyproline was isolated and measured by reverse-phase HPLC after derivatization with 7-chloro-4-nitrobenz-2-oxa-1,3-diazole. Dried hydrolysates were reconstituted in 100 μl of H_2O, buffered with 100 μl of 0.4 M potassium tetraborate and reacted with 100 μl of 12 mM NBD-Cl in methanol. Samples were incubated at 37°C for 20 min in the dark. The reaction was stopped by addition of 50 μl of 1.5 M hydrochloric acid; finally, 150 μl of 167 mM sodium acetate in acetonitrile (26% vol/vol) was added. Samples were filtered (Millipore™ type GV, pore size 0.22 μm) and a 100-μl aliquot was loaded onto the column and eluted with an acetonitrile gradient. The hydroxyproline content in each sample was determined by comparing peak areas of samples from the chromatogram with those generated from standard solutions. The total amount of collagen in each lung was calculated, assuming that lung collagen contains 12.2% w/w hydroxyproline, and expressed as mg collagen/lung.

Collagen production – fibroblasts

Cells isolated from mouse lungs were grown to confluence in 2.4 cm diameter wells in DMEM supplemented with 10% FCS and preincubated in DMEM supplemented with 1% FCS, 50 mg/ml ascorbic acid and 0.2 mM proline at 37°C for 24 h. The cell layer was scraped into the culture medium and the contents of each well collected. Wells were washed with 1 ml PBS and combined with the original medium. Proteins were precipitated by the addition of ethanol to a final concentration of 67% at 4°C overnight. Precipitated proteins were separated from free amino acids by filtration through a 0.45 mm pore-size filter and hydrolyzed in 6 N HCl at 110°C for 16 h.

Collagen promoter activity

Murine embryonic fibroblasts (MEF) derived from +/+, 757F/757F and 757F/757F;Stat3+/- mice were seeded into 24 well plates at a density of 5x10^4 cells per well in triplicate and grown overnight, 9 wells per genotype. Cells were transfected using fugene 6 transfection reagent (Roche™, manufacturer's protocol) with plasmid vector pGL-alpha, encoding the firefly luciferase under the regulation of mouse collagen alpha-1 type 1 proximal promoter (Col1a1 -2.4kb), and a plasmid vector encoding renilla luciferase under the regulation of the cytomegalovirus promoter (pCMV-RL) as a transfection control. Cells were transfected with 200ng pGL-alpha and 33ng pCMV-RL per well at atransfection reagent to DNA ratio of 6:1. In parallel plates MEFs were transfected, in triplicate, with a plasmid vector encoding firefly luciferase under the regulation of the STAT3 specific response element APRE (pAPRE-luc) and the transfection control pCMV-RL, this was to assess that the introduced gp130 mutations were preserved in MEFs and were altered due to repeated passage in vitro. Cells were transfected with 100ng pAPRE-luc and 33ng pCMV-RL per well at a transfection reagent to DNA ratio of 6:1. Transfection reaction was incubated under standard growth conditions (10% FCS, DMEM, L-glutamine, Antibiotic/Antimycotic) for 1 hour, the designer cytokine Hyper-IL-6 was added to cells at concentrations of 0ng/ml, 30ng/ml and 100ng/ml, and the cells were incubated for a further 48 hours. Luciferase activity was measured using the dual-luciferase reporter system (Promega™) as described by the manufacturer. Briefly, 100ul of passive lysis buffer was added to each well and 50ul was transferred to a 500ul tube and stored at -80C and the remaining 50ul was transferred to a 96 well plate for analysis. Luminescence of firefly luciferase was measured and 100ul of Stop & Glo™ reagent was added per well and renilla luciferase was luminescence was measured.

EXAMPLES

EXAMPLE 1 - Levels of Activated STAT3 are Reduced in Fast Growing IPF-Fibroblasts and Elevated in Slow Growing IPF-Fibroblasts

Fibroblasts isolated from normal and IPF lung tissues were characterized for growth rate. Fibroblasts isolated from normal lung tissue, hereafter referred to as "normal fibroblasts" had a doubling time of 34.38 hours. Fibroblasts isolated from different areas

of IPF lung tissue had different doubling times. One cell line isolated from IPF lung tissue, hereafter referred to as "fast growing IPF fibroblasts", had a doubling time of 41.06 hours. A different cell line isolated from IPF lung tissue, hereafter referred to as "slow growing IPF-fibroblasts", had a doubling time of 133.46 hours.

The activation of STAT3 by the cytokine interleukin-6 (IL-6) was measured in the different fibroblast cell lines. Western blots (data not shown) showed the differential activation of STAT3 (to pSTAT3) of the normal, slow growing IPF, and fast growing IPF fibroblasts, in response to IL-6. When compared to normal fibroblasts, the fast growing IPF fibroblasts had lower levels of activated STAT3 between 0 and 6 hours after application of IL-6. The difference in STAT3 activation between these cell types was most evident at the 10 minute measurement point, and was also evident at the 30 minute measurement point. When compared to normal fibroblasts, the slow growing IPF fibroblasts had higher levels of activated STAT3 (to pSTAT3), in response to IL-6. This effect was most prominent at the 10 minute and 30 minute measurement points. Figure 1 shows the change in the level of activated STAT3 in the different cell types after treatment with IL-6, as compared to untreated cells.

EXAMPLE 2 - Constitutive Activation of STAT3 in Normal-Fibroblasts Produces Hypertrophic Phenotype Resembling Slow Growing IPF-Fibroblasts

Human adult fibroblasts were transfected with constitutively active STAT3 (STAT3C). Figure 2 shows the morphological changes to the fibroblasts with constitutively active STAT3, hereafter referred to as STAT3C-fibroblasts, as compared to the morphological phenotypes of fast growing IPF fibroblasts and slow growing IPF fibroblasts. The hypertrophic phenotype of the STAT3C-fibroblasts (Figure 2B) appears very similar to the hypertrophic phenotype of the slow growing fibroblasts (Figure 2D). These hypertrophic cells appear much larger with extensive filapodia and have a web-like, fibrous appearance. There is also evidence of organized contractile filaments, which on the basis of immunofluorescence microscopy is alpha-smooth muscle actin. The fast growing IPF fibroblasts (Figure 2A) look similar to the normal fibroblast control cells (Figure 2C). Infection of cells with with an empty vector does not change the appearance of the fibroblasts. These cells do not appear hypertrophic, and do not display a significant web-like appearance or evidence of organized contractile filament formation.

EXAMPLE 3 - Collagen expression and deposition in the lung is dependent on activated STAT3

Transgenic mice with altered capacity to activate STAT3 via gp130 were created. One mouse line was created in which the expressed gp130 protein was truncated to prevent STAT3 binding. This mouse expressed lower levels of activated STAT3 and none in response to gp130 activation. A second mouse line was created which was homozygous for a mutation in the tyrosine residue at position 757 of the gp130 protein. This mutation, referred to as $gp130^{757F}$ and also as 757F, caused hyperactivation of STAT3. A third mouse line was created that was homozygous for the 757F mutation and heterozygous for STAT3 knockout ($gp130^{757F}$/ $gp130^{757F}$:STAT3+/-).

The expression of the collagen α1 gene was measured in each of the mouse lines after challenge with bleomycin, a stimulator of lung fibrosis. Deposition of total lung collagen was also estimated in each of the mouse lines after bleomycin challenge, by measurement of hydroxyproline levels in the lung. Figure 3 shows that reduction of STAT3 activation caused a reduction in expression of collagen α1 (Figure 3B) mRNA, as well as a reduction in total collagen deposition in the lung (Figure 3A), after bleomycin challenge. Hyperactivation of STAT3, caused a significant increase in collagen α1 mRNA expression (Figure 3B) and total lung collagen deposition (Figure 3A). The $gp130^{757F}$/ $gp130^{757F}$:STAT3+/- mice did not exhibit significant differences in collagen mRNA expression or deposition compared to wild type mice.

EXAMPLE 4 - STAT3 Promotes Fibrosis in Mouse Lung

Wild-type mice as well as transgenic mice with altered capacity to activate STAT3 were treated with bleomycin to induce fibrosis. Lung tissue was extracted and visualized by staining and observation by light microscopy. Micrographs of lung tissue from different mouse lines were examined (data not shown). Masson's trichrome stain for collagen (blue stain) revealed mild thickening of alveolar septa and collagen deposition 30 days after bleomycin administration in the lungs of wild-type mice. However, marked attenuation in accumulated collagen was observed in mice with reduced ability to activate STAT3 (ΔSTAT mice). In contrast, dense fibrosis with prominent collagen deposition was observed 30 days after bleomycin administration in the lungs of 757F mice. No abnormal alveolar architecture was observed in the lungs of the saline-administered control group in

either wild-type mice or transgenic mice. The lung hydroxyproline content was measured and shown to be significantly increased in wildtype mice 30 days after bleomycin exposure. Hydroxyproline levels were not different in ΔSTAT mice following bleomycin exposure. In 757F mice, levels of hydroxyproline were significantly increased compared to saline challenge and were also significantly greater than levels in wildtype mice following bleomycin (Figure 4).

EXAMPLE 5 - STAT3 Increases Collagen α1 Promoter Activity in Lung Fibroblasts

Figure 5 shows a bar graph demonstrates collagen α1 promoter activity in lung fibroblasts derived from wild-type (WT), hyper-activated STAT3 ($gp130^{757F}$), and STAT3 defective ($gp130^{757F}/gp130^{757F}$:$STAT3^{+/-}$) animals in the presence of gp130 activation ligand – IL-6, at different concentrations. The X-axis represents the concentrations (ng/ml) of recombinant human interleukin 6 used to activate the cells while the Y-axis depicts the ratio of firefly luciferase activity driven by the collagen α1 promoter, compared to the activity of the internal control Renilla luciferase. **P<0.05 compared with WT and $gp130^{757F}$x$STAT3^{+/-}$ fibroblasts.

EXAMPLE 6 - Gene Expression Activity in Lung Fibroblasts

TABLE 4 shows a number of genes from fibroblast cells that were compared between control fibroblasts and slow growing IPF fibroblasts (*p<0.05 compared with Control-FBR). Those genes associated with cellular senescence are underlined.

TABLE 4

Genes	Control-FBR	IPF-FBR
FBN1 (fibrillin-1)	156.9±14.6	299.9±46.1*
FN1 (fibronectin)#	19 022.7±1 767.5	36 437.3±8 810.2*
COL1A1 (collagen 1a1)	2 297.3±624.8	3 008.8±940.4*
PCOLN3 (pro-collagen 3)	37.8±2.6	47.3±2.4
CTGF (connective tissue growth factor)	1 293.2±185.6	1 668.5±493.1
TGFB1 (transforming growth factor beta 1)	221.2±48.4	452.6±27.7*
IL6 (interleukin-6)	48.5±8.4	85.1±34.4*
TGFBR1 (TGF beta receptor 1)	166.0±23.8	89.0±25.6*

PDGFRA (platelet-derived growth factor receptor alpha)	568.9±105.9	365.7±72.1
PDGFRB (platelet-derived growth factor receptor beta)	265.3±42.3	107.2±10.3*

EXAMPLE 7 - STAT3C induced expression of collagen in human lung fibroblasts.

Figure 6 shows spontaneous and TGFβ1-induced expression of collagen 1α1 transcripts in 2 different lines of human lung fibroblasts, non-infected and infected with STAT3C or LacZ lentivirus expressing constructs.

EXAMPLE 8 - Gp130-STAT1/3 signaling promotes fibrosis independent of Smad3.

Figure 7 shows STAT3 activation regulates lung fibrosis and collagen synthesis independent of Smad3. The role of Smad3 signalling in the development bleomycin-induced fibrosis assessed by treating Smad3 null mice ($Sm3^{-/-}$) and gp130757F/757F mice cross-bred with Smad3 null mice (F/F; $Sm3^{-/-}$) with bleomycin. Fibrosis was assessed by Masson's trichrome stain of lung tissue and total collagen accumulation 21 days after bleomycin treatment. $Sm3^{-/-}$ had no signs of fibrosis 21 days after bleomycin treatment, in contrast F/F; $Sm3^{-/-}$ mice showed extensive fibrosis of the lung parenchyma 21 days after bleomycin treatment.

EXAMPLE 9 - Proliferation is slowed dramatically in cells with constitutively high STAT3 activity.

Figure 8 shows relative proliferation rates of fibroblasts were determined using the Cell Proliferation Biotrak ELISA System™, version 2 (Amersham Biosciences™, Piscataway, NJ), according to the manufacturer's directions. Cells were seeded in a 96-well plate at a density of 5,000 cells per well. Cells were quiesced in serum-free media for 24 hours prior to stimulation, and BrdU was added at 24 hours post stimulation.

EXAMPLE 10 - STAT3 Inhibition by STA-21

Non-peptide low molecular weight compound STA-21 inhibits Stat3 DNA binding activity, Stat3 dimerization, and has been reported to reduce the survival of breast carcinoma cells with constitutive Stat3 signaling (Song et al. 2005).

However, the effect of STA-21 on Stat3 signaling in human primary lung fibroblasts is unknown. Using "in vitro" cultures of human fibroblasts isolated from bronchial and lung tissue biopsies we determined effects of the Stat3 inhibitor STA-21 on spontaneous and epidermal growth factor (EGF)- and oncostatin M (OSM)-induced STAT3 signaling.

Preliminary results suggest that STA-21 displays Stat3 inhibitory activity in human primary bronchial and lung fibroblasts. Figure 9A demonstrates the inhibitory effect of STA-21 on OSM-induced SOCS3 expression in bronchial fibroblasts showing high level of spontaneous Stat3 phosphorylation (activation)). On the other hand, STA-21 significantly increased beta 3-integrin expression in the same cells (Fig.9B). It was found that lung fibroblasts isolated from patients with IPF are deficient in beta3-integrin expression and this deficiency is associated with Stat3-signaling activation (Pechkovsky *et al.* unpublished observation). Furthermore, Figure 10 shows that STA-21 attenuates the up-regulation of EGF and OSM on OSM-(β)-receptor and IL-6-(α)-receptor expression in human primary lung fibroblasts. STA-21 also prevents OSM-induced Stat3 expression in human primary bronchial (A) and lung (B) fibroblasts (see Figure 11). Furthermore, Stat3 inhibitors Cucurbitacine Q and STA-21 do not influence on Tyr(P)705-Stat3 induced by OSM or IL-6 in human lung fibroblasts (results not shown).

EXAMPLE 11 - STAT3 Inhibition by siRNA

STAT3 siRNA showed dose dependent inhibition of STAT3 expression in primary lung fibroblasts isolated from IPF lung tissue. IPF fibroblasts were transfected with siRNA (STAT3 siRNA was produced by Dharmacon™ and is a "smartpool" of 4 different siRNA's) for STAT3 and two days later total RNA was collected, STAT3 expression evaluated by real-time RT-PCR (see Figure 12). STAT3 siRNA validation – primary lung fibroblasts isolated from IPF lung tissue (138-IPF) were transfected with siRNA for STAT3.

EXAMPLE 12 - Constitutively active Stat3 (STAT3C) expressed in primary human lung fibroblast represses Thy-1 (CD90) cell surface expression.

THYmocyte differentiation antigen 1 (Thy-1; THY1; T-CELL ANTIGEN; THETA ANTIGEN; CD90 ANTIGEN; CD90) is a major cell surface glycoprotein and has reduced or absent expression of Thy-1 is generally accepted to be a marker of a

myofibroblast. STAT3C completely abrogates Thy-1 expression in non-IPF fibroblasts (see Figure 13). Representative flow cytometry histograms are shown in Figure 13(**A**). Thy-1 expression significantly decreased in HLF-STAT3C transduced cells in comparison with non-infected (HLF-Ctrl) and LacZ transduced cells (HLF-LacZ). Figure 13(**B**) shows screening of 14 individual lines of human lung fibroblasts isolated from lung tissue samples and anatomical locations for Thy-1 expression and basal levels of Stat3 activation revealed a negative correlation between percentage of Thy-1 positive cells and levels of spontaneous Stat3 posphorylation in the given cell population. STAT-3C reduces expression of Thy-1. Thy-1 expression is reduced or absent and is generally accepted to be a marker of a myofibroblast. STAT3C completely abrogates Thy-1 expression in non-IPF fibroblasts.

REFERENCES

Boulianne, G. L. *et al.*, (1984) Nature 312:643-646.

Bromberg, J. F., M. H. Wrzeszczynska, *et al.* (1999). "Stat3 as an oncogene." Cell 98(3): 295-303.

Burton D. R. (1991) Proc. Natl. Acad. Sci. 88:11120-3.

Chung, C. D., J. Liao, *et al.* (1997). "Specific inhibition of Stat3 signal transduction by PIAS3." Science 278(5344): 1803-5.

Cole, S. P. *et al.* (1984) Mol. Cell Biol. 62:109-120.

Coleman, D. R. T., Z. Ren, *et al.* (2005). "Investigation of the binding determinants of phosphopeptides targeted to the SRC homology 2 domain of the signal transducer and activator of transcription 3. Development of a high-affinity peptide inhibitor." J Med Chem 48(21): 6661-70.

Cote, R. J. *et al.* (1983) Proc. Natl. Acad. Sci. 80:2026-2030.

Dechow, T. N., L. Pedranzini, *et al.* (2004). "Requirement of matrix metalloproteinase-9 for the transformation of human mammary epithelial cells by Stat3-C." Proc Natl Acad Sci U S A 101(29): 10602-7.

Desmouliere, A., C. Chaponnier, *et al.* (2005). "Tissue repair, contraction, and the myofibroblast." Wound Repair Regen 13(1): 7-12.

Ernst, M., M. Inglese, *et al.* (2001). "Defective gp130-mediated signal transducer and activator of transcription (STAT) signaling results in degenerative joint disease, gastrointestinal ulceration, and failure of uterine implantation." J Exp Med 194(2): 189-203.

Gao H. and Ward PA. (2007). "STAT3 and suppressor of cytokine signaling 3: potential targets in lung inflammatory responses." Expert Opin. Ther. Targets 11(7):869-880.

Hodge, D. R., E. M. Hurt, *et al.* (2005). "The role of IL-6 and STAT3 in inflammation and cancer." Eur J Cancer 41(16): 2502-12.

Huang J-S. *et al.* (1999) "Role of the Janus kinase (JAK)/signal transducters and activators of transcription (STAT) cascade in advanced glycation end-product-induced cellular mitogenesis in NRK-49F cells." Biochem. J. 342:231-238.

Huse, W. D. *et al.* (1989) Science 254:1275-1281.

Jordana, M., J. Schulman, *et al.* (1988). "Heterogeneous proliferative characteristics of human adult lung fibroblast lines and clonally derived fibroblasts from control and fibrotic tissue." Am Rev Respir Dis 137(3): 579-84.

Judd, L. M., K. Bredin, *et al.* (2006). "STAT3 activation regulates growth, inflammation, and vascularization in a mouse model of gastric tumorigenesis." Gastroenterology 131(4): 1073-85.

Kohler, G. *et al.* (1975) Nature 256:495-497.

Kozbor, D. *et al.* (1985) J. Immunol. Methods 81:31-42.

Lim, C. P., T. T. Phan, *et al.* (2006). "Stat3 contributes to keloid pathogenesis via promoting collagen production, cell proliferation and migration." Oncogene 25(39): 5416-25.

Maddox, D. E. *et al.* (1983) J. Exp. Med. 158:1211-1216.

Moodley, Y. P., N. L. Misso, *et al.* (2003a). "Inverse effects of interleukin-6 on apoptosis of fibroblasts from pulmonary fibrosis and normal lungs." Am J Respir Cell Mol Biol 29(4): 490-8.

Moodley, Y. P., A. K. Scaffidi, *et al.* (2003b). "Fibroblasts isolated from normal lungs and those with idiopathic pulmonary fibrosis differ in interleukin-6/gp130-mediated cell signaling and proliferation." Am J Pathol 163(1): 345-54.

Morgan and Foon (1984) "Monoclonal Antibody Therapy to Cancer: Preclinical Models and Investigations, Basic and Clinical Tumor Immunology", Vol. 2, Kluwer Academic Publishers, Hingham, Mass.) and by Uhr, J. of Immunol. 133:i-vii)

Morrison, S. L. *et al.* (1984) Proc. Natl. Acad. Sci. 81:6851-6855.

Neuberger, M. S. *et al.* (1984) Nature 312:604-608.

Neuberger, M. S. *et al.*, (1985) Nature 314:268-270.

Orlandi, R. *et al.* (1989) Proc. Natl. Acad. Sci. 86: 3833-3837.

Pan, W. H. and G. A. Clawson (2006). "Identifying accessible sites in RNA: the first step in designing antisense reagents." Curr Med Chem 13(25): 3083-103.

Pardo, A. and M. Selman (2002). "Molecular mechanisms of pulmonary fibrosis." Front Biosci 7: d1743-61.

Patzel, V. (2007). "In silico selection of active siRNA." Drug Discov Today 12(3-4): 139-48.

Peek, A. S. and M. A. Behlke (2007). "Design of active small interfering RNAs." Curr Opin Mol Ther 9(2): 110-8.

Ramos, C., M. Montano, *et al.* (2001). "Fibroblasts from idiopathic pulmonary fibrosis and normal lungs differ in growth rate, apoptosis, and tissue inhibitor of metalloproteinases expression." Am J Respir Cell Mol Biol 24(5): 591-8.

Selman, M., T. E. King, et al. (2001). "Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy." Ann Intern Med 134(2): 136-51.

Si HF. *et al.* (2007) "Suppressive effects of leflunomide on leptin-induced collagen I production involved in hepatic stellate cell proliferation." Exp Biol Med 232:427-436.

Simon AR. *et al.* (2002) "Role of the JAK-STAT pathway in PDGF-stimulated proliferation of human airway smooth muscle cells." Am J Physiol Lung Cell Mol Physiol 282:L1296-L1304.

Song, H., R. Wang, *et al.* (2005). "A low-molecular-weight compound discovered through virtual database screening inhibits Stat3 function in breast cancer cells." Proc Natl Acad Sci U S A 102(13): 4700-5.

Stärkel P. *et al.* (2007) "Role of signal transducer and activator of transcription 3 in liver fibrosis progression in chronic hepatitis C-infected patients." Lab Invest 87:173-181.

Takeda, K., K. Noguchi, *et al.* (1997). "Targeted disruption of the mouse Stat3 gene leads to early embryonic lethality." Proc Natl Acad Sci U S A 94(8): 3801-4.

Takeda S. *et al.* (1985) Nature 314:452-454.

Tebbutt, N. C., A. S. Giraud, et al. (2002). "Reciprocal regulation of gastrointestinal homeostasis by SHP2 and STAT-mediated trefoil gene activation in gp130 mutant mice." Nat Med 8(10): 1089-97.

Walters DM. *et al.* (2005) "Susceptibility of signal transducer and activator of transcription-1-deficient mice to pulmonary fibrogenesis." Am J Pathol 167(5):1221-1229.

Wang, L., S. Tanaka, *et al.* (2005). "GATA-4 binds to an upstream element of the human alpha2(I) collagen gene (COL1A2) and inhibits transcription in fibroblasts." Matrix Biol 24(5): 333-40.

Winter G. *et al.* (1991) Nature 349:293-299.

Wu SH. *et al.* (2006) "Lipoxin A4 inhibits proliferation of human lung fibroblasts induced by connective tissue growth factor." Am J Respir Cell Mol Biol 34:65-72.

WHAT IS CLAIMED IS:

1. A method of preventing or treating an interstitial lung disease (ILD) in a subject in need thereof, the method comprising administering to the subject a signal transducer and activator of transcription 3 (STAT3) inhibitor.

2. The method of claim 1, wherein STAT3 inhibitor is selected from one or more of: a STAT3 peptidomimetic; Protein Inhibitor of Activated STAT3 (PIAS3); a STAT3 antisense RNA; a STAT3 siRNA; Lipoxin A_4 (LXA_4); LY294002; STA-21; Leflunomide; 6-nitro-1-benzothiophene 1,1-dioxide (CID: 2779853); Cucurbitacin D; Tetrahydro-Cucurbitacin I; NSC628869; NSC94743; NSC49451; NSC521777; NSC106399; and NSC135075; analogues thereof; variants thereof; pharmaceutically acceptable salts thereof; a pro-drugs thereof; a metabolites thereof; and a pharmaceutical composition comprising one or more of the above.

3. The method of claim 2, wherein the antisense RNA corresponds to SEQ ID NO:1.

4. The method of claim 1 or 2, wherein the STAT3 inhibitor is STA-21.

5. The method of claim 1 or 2, wherein the STAT3 inhibitor is PIAS3.

6. The method of claim 1 or 2, wherein the STAT3 inhibitor is LXA4.

7. The method of claim 1 or 2, wherein the STAT3 inhibitor is LY294002.

8. The method of claim 1 or 2, wherein the STAT3 inhibitor is 6-nitro-1-benzothiophene 1,1-dioxide.

9. The method of claim 1 or 2, wherein the STAT3 inhibitor is NSC94743.

10. The method of claim 1 or 2, wherein the STAT3 inhibitor is NSC628869.

11. The method of claim 1 or 2, wherein the STAT3 inhibitor is NSC49451.

12. The method of claim 1 or 2, wherein the STAT3 inhibitor is NSC521777.

13. The method of claim 1 or 2, wherein the STAT3 inhibitor is NSC106399.

14. The method of claim 1 or 2, wherein the STAT3 inhibitor is NSC135075.

15. The method of claim 1 or 2, wherein the STAT3 inhibitor is Leflunomide.

16. The method any one of claims 1-15, further comprising targeting slow growing, differentiated, hypertrophic fibroblasts in fibrotic lung tissue.

17. The method of claim 16, wherein the targeting comprises coupling the STAT3 inhibitor to a targeting moiety.

18. The method of claim 17, wherein the targeting moiety is an antibody having specificity for one or more of the following: fibronectin (FN1); collagen 1α1 (COL1A1);

α-smooth muscle actin (α-SMA); connective tissue growth factor (CTFG); transforming growth factor beta 1 (TGFB1); and interleukin-6 (IL-6).

19. The method of claim 18, wherein the antibody is humanized.

20. The method of any one of claims 1-19, wherein the STAT3 inhibitor is formulated for inhalation.

21. The method of any one of claims 1-19, wherein the STAT3 inhibitor is formulated for administration by injection.

22. The method of any one of claims 1-19, wherein the STAT3 inhibitor is formulated for oral administration.

23. The method of any one of claims 1-19, wherein the STAT3 inhibitor is formulated for topical administration.

24. The method of any one of claims 1-23, wherein the subject is a human.

25. The method of any one of claims 1-24, wherein the ILD is fibrotic lung disease.

26. The method of any one of claims 1-25, wherein the ILD is selected from one or more of: idiopathic pulmonary fibrosis (IPF); pulmonary fibrosis (PF); acute lung injury; hypersensitivity pneumonitis; and drug-induced lung fibrosis.

27. The method of any one of claims 1-26, wherein the ILD is IPF.

28. Use of a STAT3 inhibitor in the manufacture of a medicament for the prevention or treatment of an ILD in a subject in need thereof.

29. Use of a STAT3 inhibitor for the prevention or treatment of an ILD in a subject in need thereof.

30. Use of a pharmaceutical composition comprising a STAT3 inhibitor for the prevention or treatment of an ILD in a subject in need thereof.

31. The use of any one of claims 28-30, wherein STAT3 inhibitor is selected from one or more of: a STAT3 peptidomimetic; Protein Inhibitor of Activated STAT3 (PIAS3); a STAT3 antisense RNA; a STAT3 siRNA; Lipoxin A_4 (LXA_4); LY294002; STA-21; Leflunomide; 6-nitro-1-benzothiophene 1,1-dioxide (CID: 2779853); Cucurbitacin D; Tetrahydro- Cucurbitacin I; NSC628869; NSC94743; NSC49451; NSC521777; NSC106399; and NSC135075; analogues thereof; variants thereof; pharmaceutically acceptable salts thereof; a pro-drugs thereof; a metabolites thereof; and a pharmaceutical composition comprising one or more of the above.

32. The use of claim 31, wherein the antisense RNA corresponds to SEQ ID NO:1.

33. The use of any one of claims 28-31, wherein the STAT3 inhibitor is STA-21.

34. The use of any one of claims 28-31, wherein the STAT3 inhibitor is PIAS3.

35. The use of any one of claims 28-31, wherein the STAT3 inhibitor is LXA4.

36. The use of any one of claims 28-31, wherein the STAT3 inhibitor is LY294002.

37. The use of any one of claims 28-31, wherein the STAT3 inhibitor is 6-nitro-1-benzothiophene 1,1-dioxide.

38. The use of any one of claims 28-31, wherein the STAT3 inhibitor is NSC94743.

39. The use of any one of claims 28-31, wherein the STAT3 inhibitor is NSC628869.

40. The use of any one of claims 28-31, wherein the STAT3 inhibitor is NSC49451.

41. The use of any one of claims 28-31, wherein the STAT3 inhibitor is NSC521777.

42. The use of any one of claims 28-31, wherein the STAT3 inhibitor is NSC106399.

43. The use of any one of claims 28-31, wherein the STAT3 inhibitor is NSC135075.

44. The use of any one of claims 28-31, wherein the STAT3 inhibitor is Leflunomide.

45. The use of any one of claims 28-44, further comprising targeting slow growing, differentiated, hypertrophic fibroblasts in fibrotic lung tissue.

46. The use of claim 45, wherein the targeting comprises coupling the STAT3 inhibitor to a targeting moiety.

47. The use of claim 46, wherein the targeting moiety is an antibody having specificity for one or more of the following: fibronectin (FN1); α-smooth muscle actin (α-SMA); collagen 1α1 (COL1A1); connective tissue growth factor (CTFG); transforming growth factor beta 1 (TGFB1); and interleukin-6 (IL-6).

48. The use of claim 47, wherein the antibody is humanized.

49. The use of any one of claims 28-48, wherein the STAT3 inhibitor is formulated for inhalation.

50. The use of any one of claims 28-48, wherein the STAT3 inhibitor is formulated for administration by injection.

51. The use of any one of claims 28-48, wherein the STAT3 inhibitor is formulated for oral administration.

52. The use of any one of claims 28-48, wherein the STAT3 inhibitor is formulated for topical administration.

53. The use of any one of claims 28-52, wherein the subject is a human.

54. The use of any one of claims 28-53, wherein the ILD is fibrotic lung disease.

55. The use of any one of claims 28-54, wherein the ILD is selected from one or more of: idiopathic pulmonary fibrosis (IPF); pulmonary fibrosis (PF); acute lung injury; hypersensitivity pneumonitis; and drug-induced lung fibrosis.

56. The use of any one of claims 28-55, wherein the ILD is IPF.

57. A commercial package comprising:

(a) a pharmaceutical composition comprising:

(i) STAT3 inhibitor; and

(ii) a pharmaceutically acceptable carrier; and

(b) instructions for the use thereof for treating an ILD.

58. A method for screening for or identifying a test compound that inhibits the production of collagen in a fibroblast, the method comprising administering the test compound or test compounds to fibroblast and assaying for decreased expression or activity of collagen or the collagen α1 promoter.

FIGURE 1

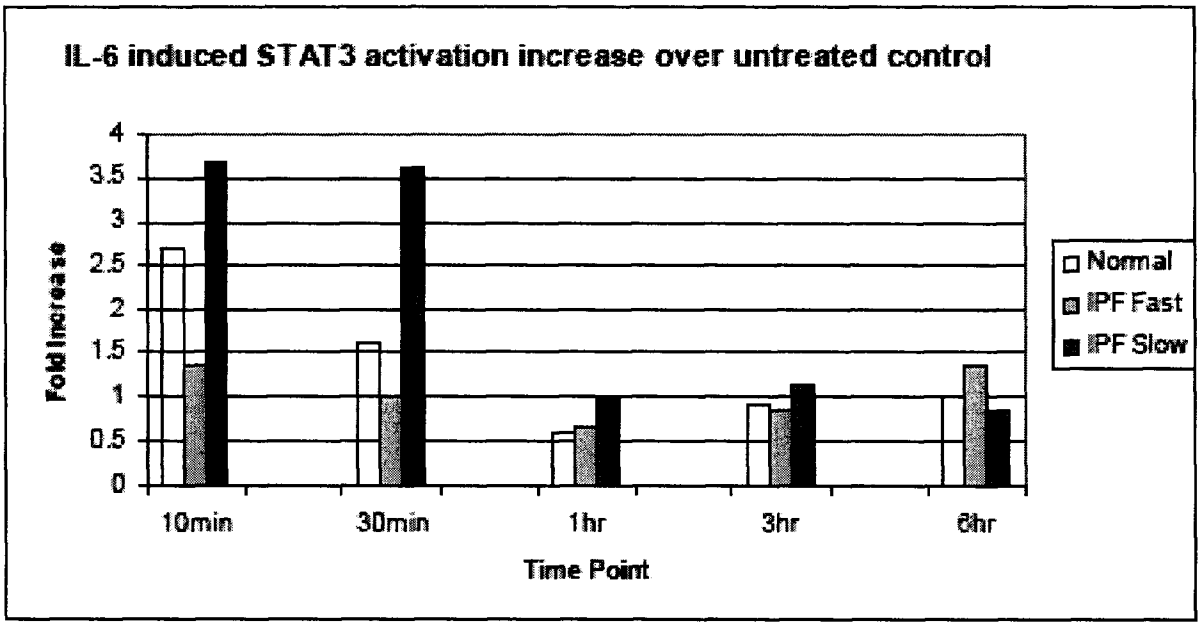

IL-6 induced STAT3 activation increase over untreated control
Fold Increase
4
3.5
3
2.5
2
1.5
1
0.5
0
10min
30min
1hr
3hr
6hr
Time Point
Normal
IPF Fast
IPF Slow

FIGURE 2

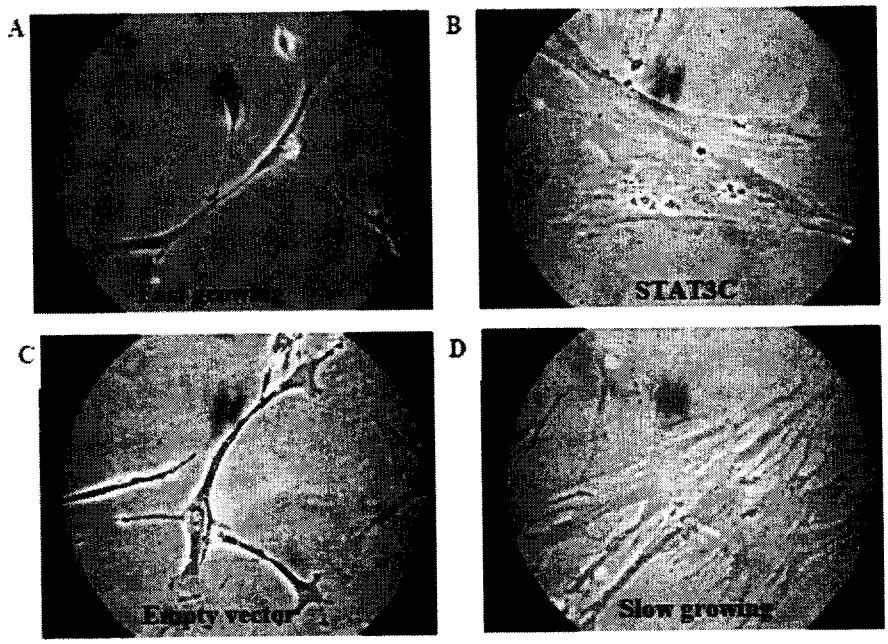


FIGURE 3

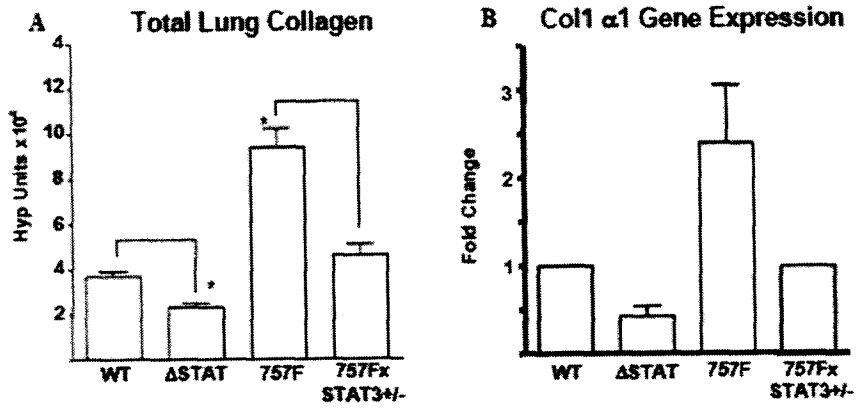

A
Total Lung Collagen
Hyp Units x 10⁴
4
12
10
8
6
4
2
*
*
WT
ΔSTAT
757F
757Fx STAT3+/-
B
Col1 α1 Gene Expression
Fold Change
3
2
1
WT
ΔSTAT
757F
757Fx STAT3+/-

FIGURE 4

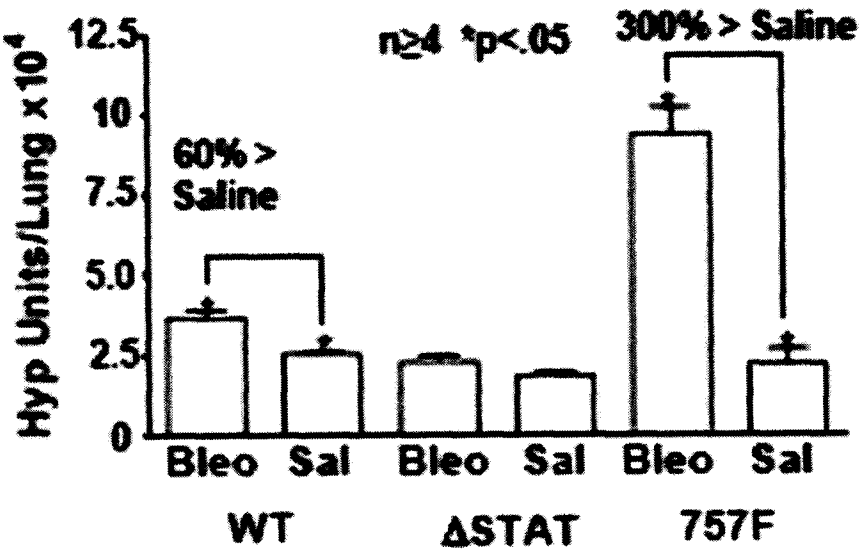

Hyp Units/Lung x 10⁴
12.5
10
7.5
5.0
2.5
0
n≥4 *p<.05
300% > Saline
60% > Saline
Bleo
Sal
Bleo
Sal
Bleo
Sal
WT
ΔSTAT
757F

FIGURE 5

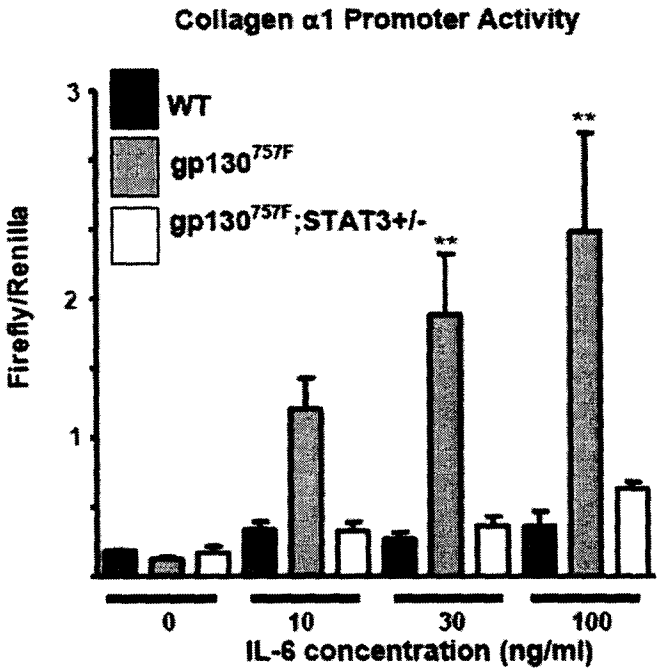

Collagen α1 Promoter Activity
WT
gp130757F
gp130757F;STAT3+/-
Firefly/Renilla
3
2
1
**
**
0
10
30
100
IL-6 concentration (ng/ml)

FIGURE 6

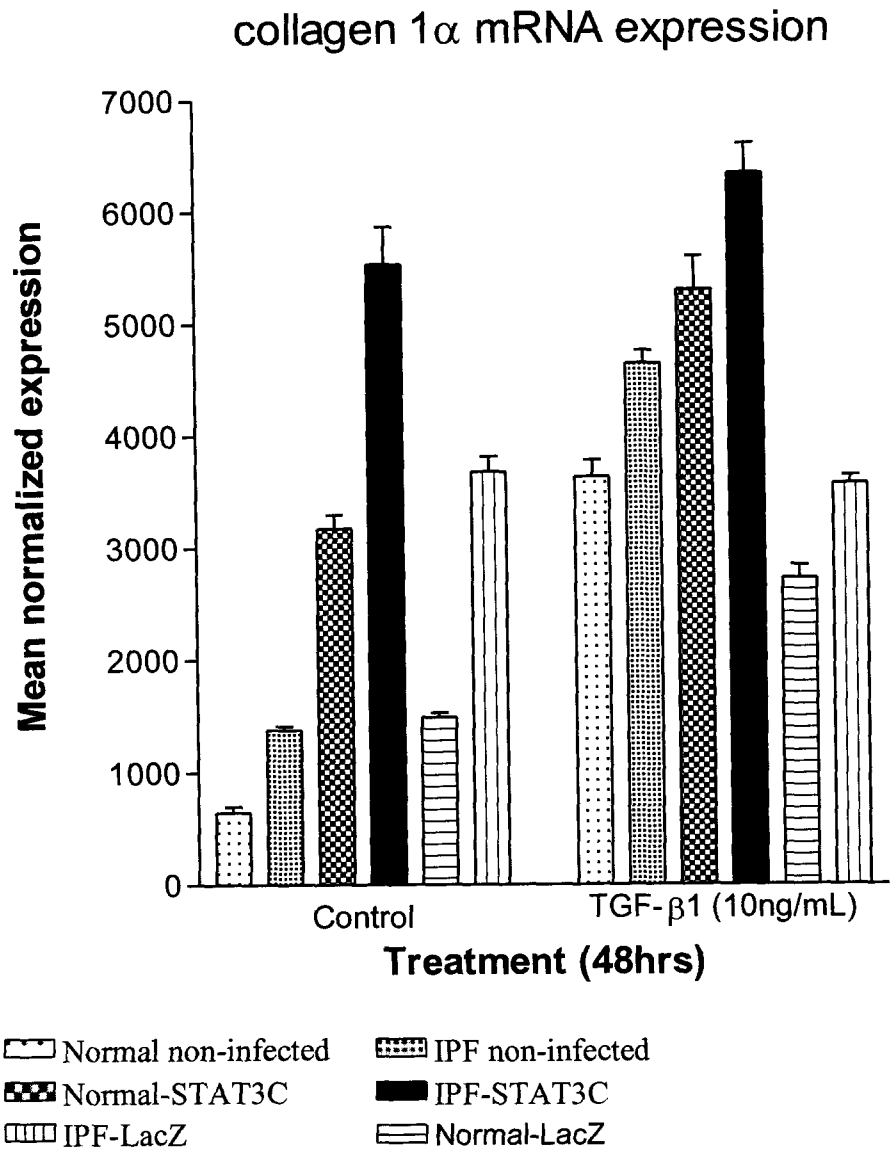

collagen 1α mRNA expression
Mean normalized expression
7000
6000
5000
4000
3000
2000
1000
0
Control
TGF-β1 (10ng/mL)
Treatment (48hrs)
Normal non-infected
IPF non-infected
Normal-STAT3C
IPF-STAT3C
IPF-LacZ
Normal-LacZ

FIGURE 7

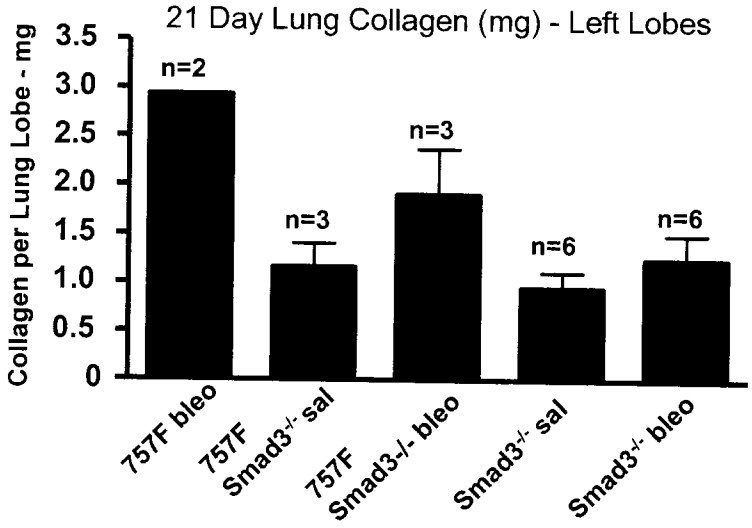

21 Day Lung Collagen (mg) - Left Lobes
Collagen per Lung Lobe - mg
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
n=2
n=3
n=3
n=6
n=6
757F bleo
757F Smad3-/- sal
757F Smad3-/- bleo
Smad3-/- sal
Smad3-/- bleo

FIGURE 8

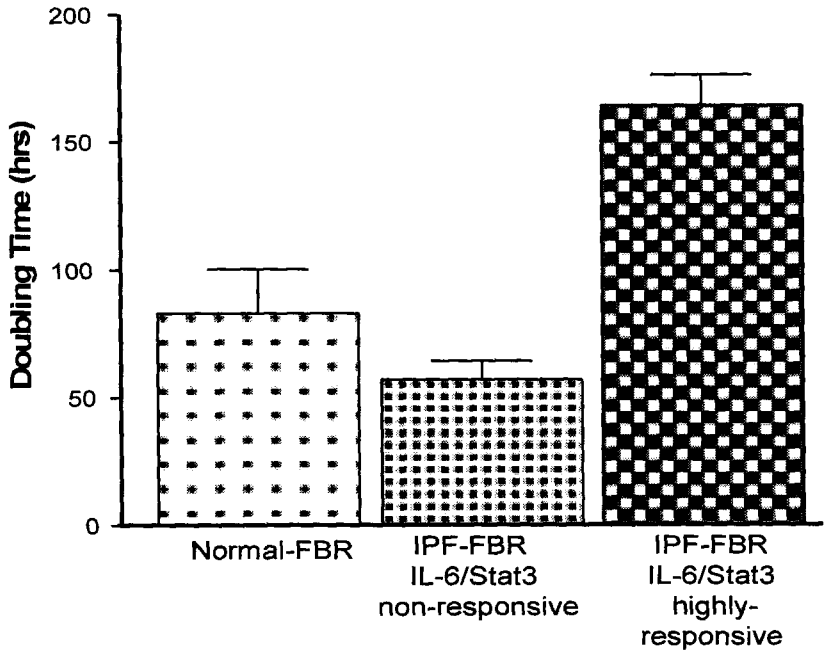

200
150
100
50
0
Doubling Time (hrs)
Normal-FBR
IPF-FBR IL-6/Stat3 non-responsive
IPF-FBR IL-6/Stat3 highly-responsive

FIGURE 9.

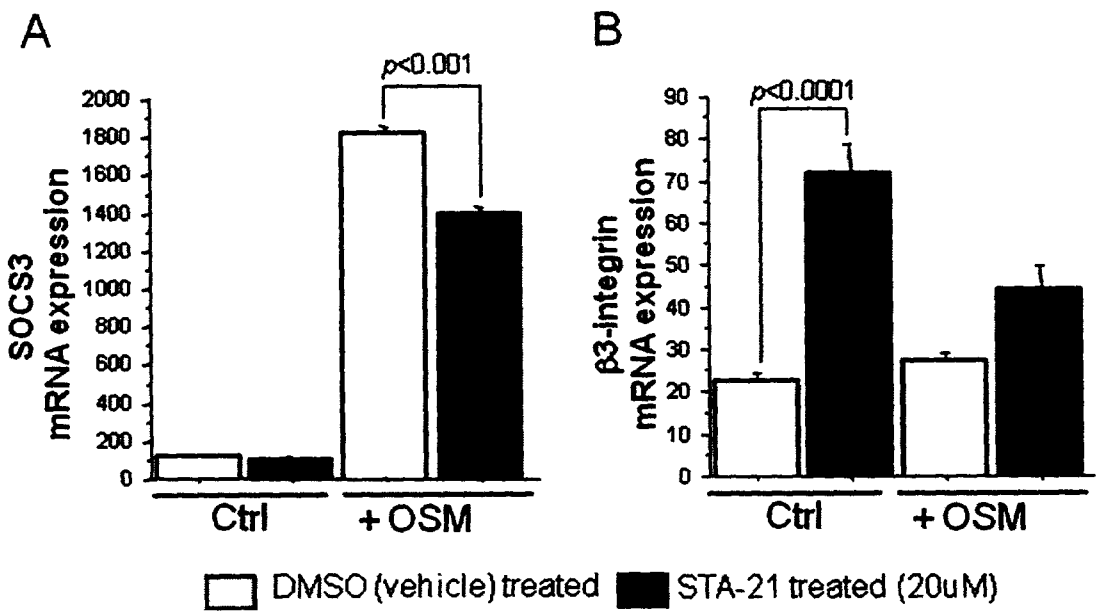

A
p<0.001
SOCS3 mRNA expression
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Ctrl
+OSM
B
p<0.0001
β3-integrin mRNA expression
90
80
70
60
50
40
30
20
10
0
Ctrl
+OSM
DMSO (vehicle) treated
STA-21 treated (20uM)

FIGURE 10.

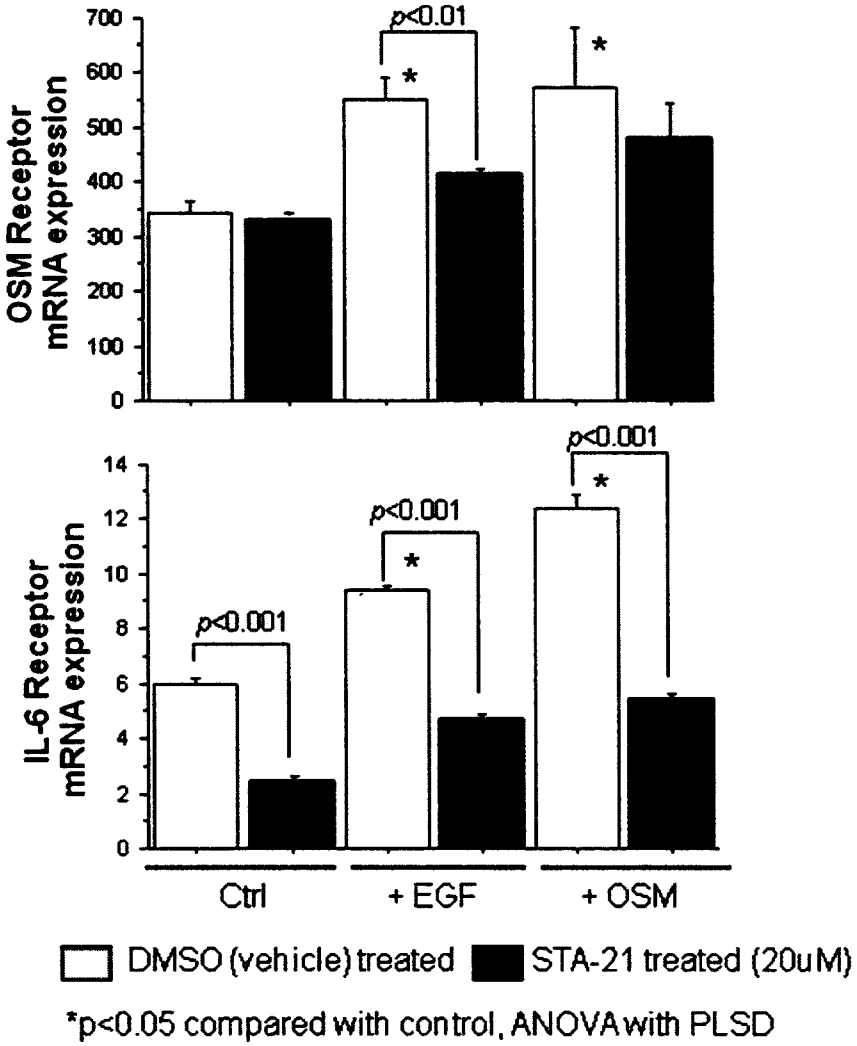

OSM Receptor mRNA expression
700
600
500
400
300
200
100
0
p<0.01
*
*
IL-6 Receptor mRNA expression
14
12
10
8
6
4
2
0
p<0.001
p<0.001
p<0.001
*
*
*
Ctrl
+ EGF
+ OSM
DMSO (vehicle) treated
STA-21 treated (20uM)
*p<0.05 compared with control, ANOVA with PLSD

FIGURE 11.

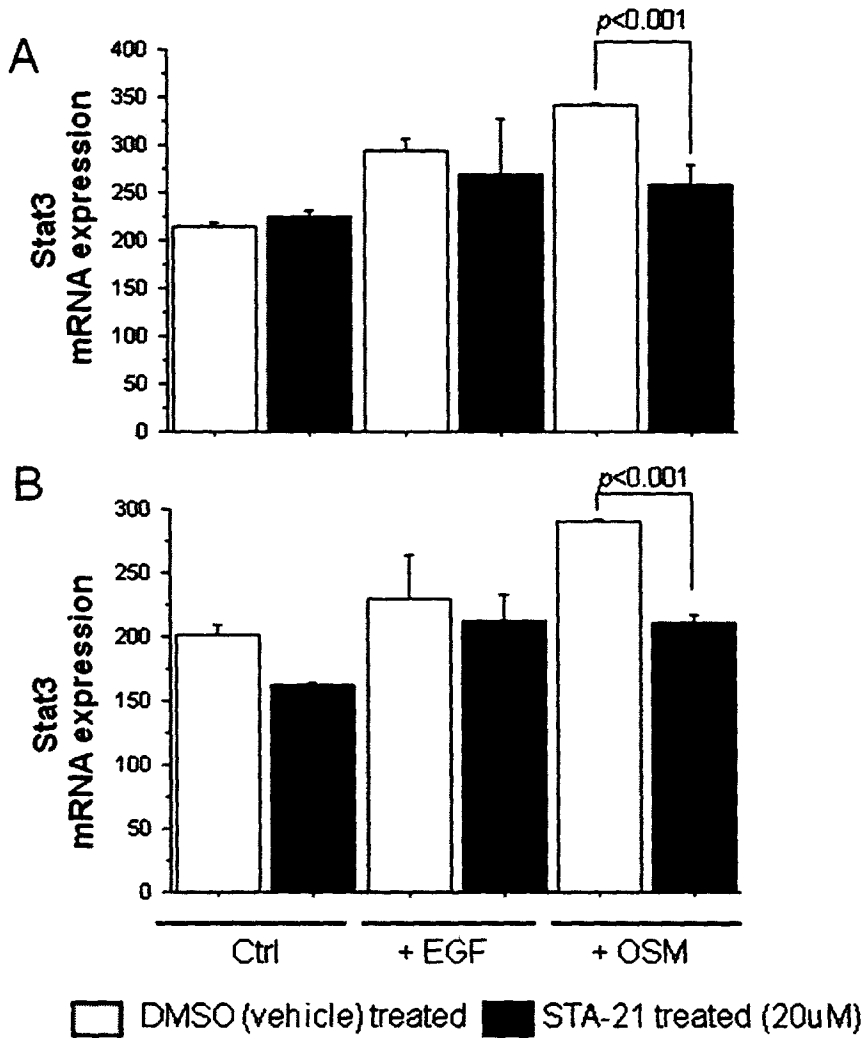

A
B
Stat3 mRNA expression
p<0.001
400
350
300
250
200
150
100
50
0
Ctrl
+ EGF
+ OSM
DMSO (vehicle) treated
STA-21 treated (20uM)

FIGURE 12.

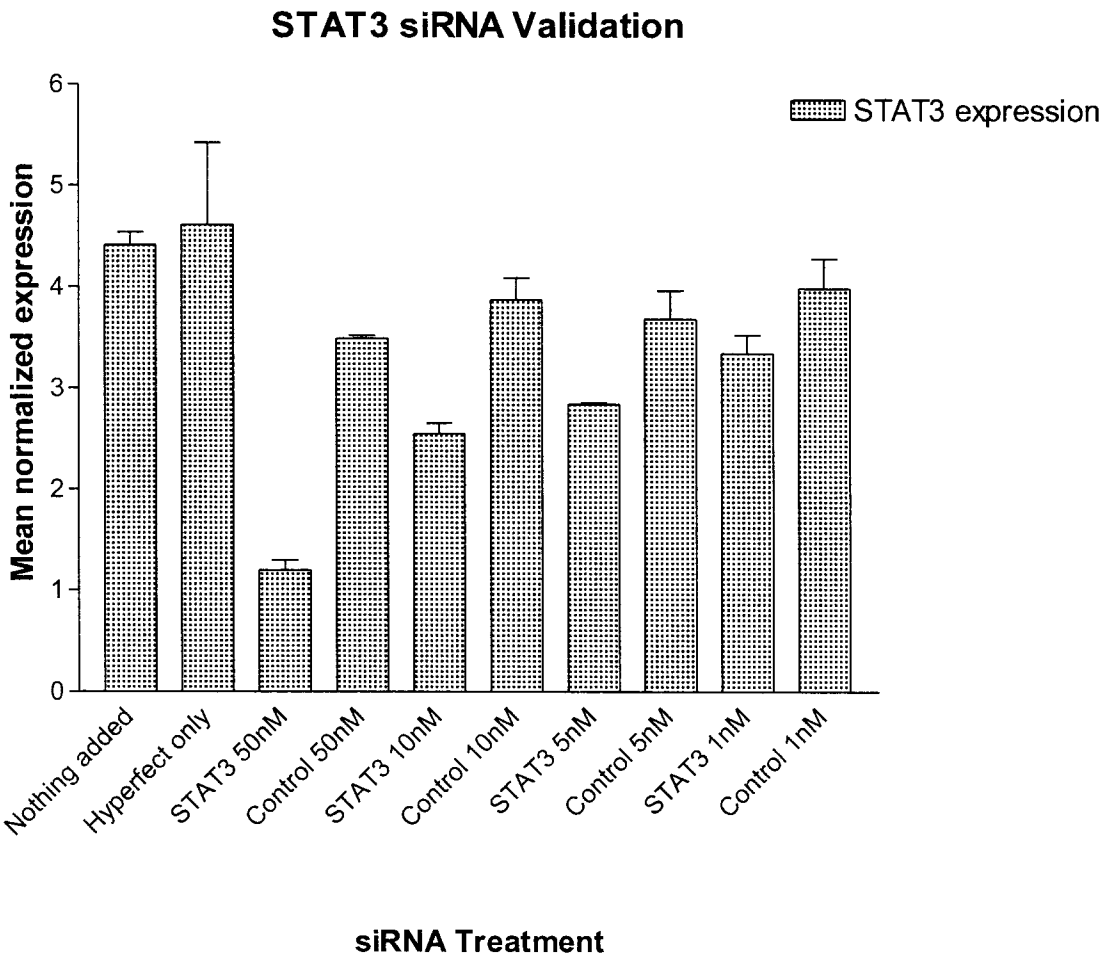
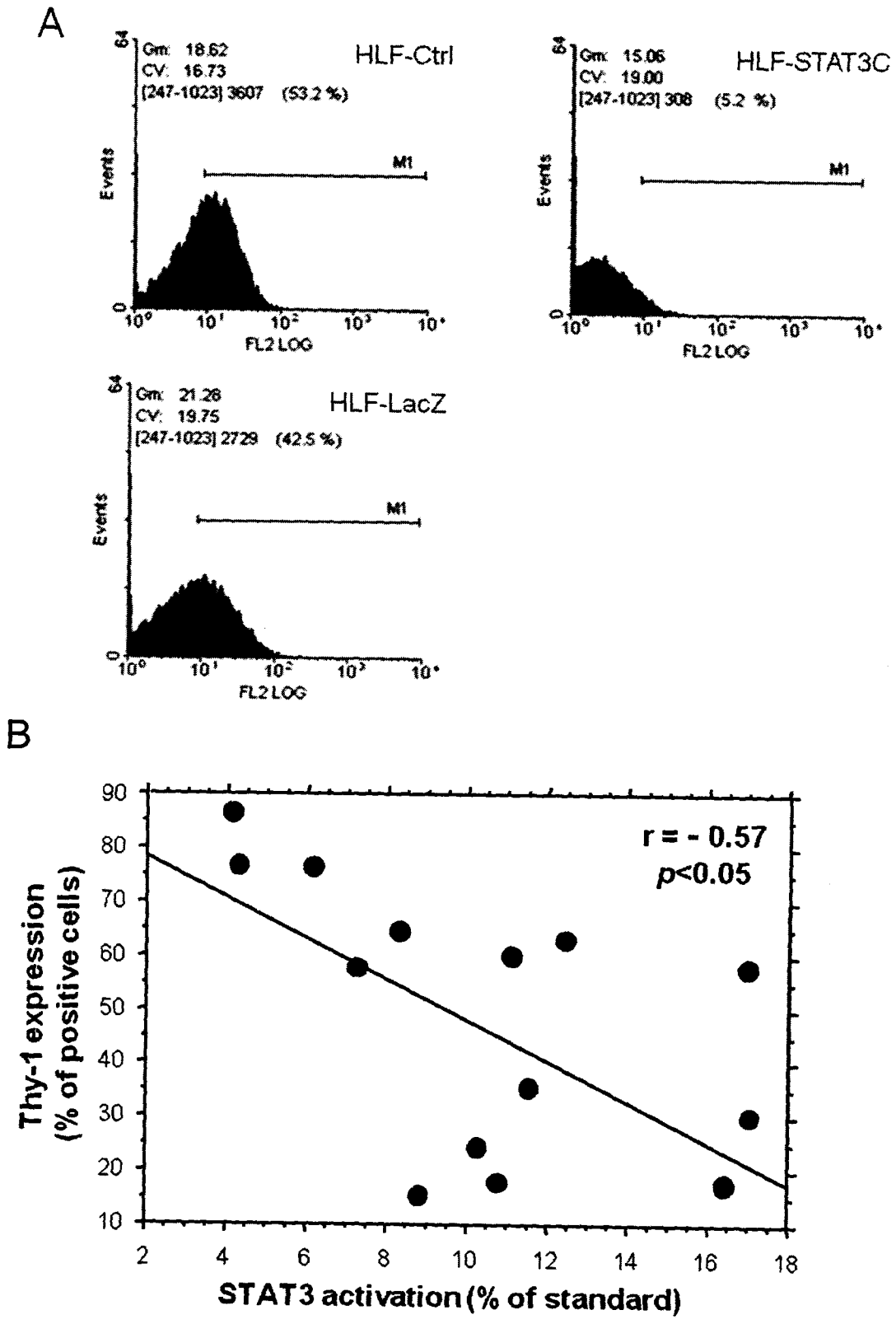

STAT3 siRNA Validation
STAT3 expression
Mean normalized expression
0
1
2
3
4
5
6
Nothing added
Hyperfect only
STAT3 50nM
Control 50nM
STAT3 10nM
Control 10nM
STAT3 5nM
Control 5nM
STAT3 1nM
Control 1nM
siRNA Treatment

FIGURE 13.


A
Gm: 18.62
CV: 16.73
[247-1023] 3607 (53.2 %)
HLF-Ctrl
Gm: 15.06
CV: 19.00
[247-1023] 308 (5.2 %)
HLF-STAT3C
Gm: 21.28
CV: 19.75
[247-1023] 2729 (42.5 %)
HLF-LacZ
M1
Events
FL2 LOG
B
r = - 0.57
p<0.05
Thy-1 expression (% of positive cells)
STAT3 activation (% of standard)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2008/001971

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***A61K 31/575*** (2006.01) , ***A61K 31/122*** (2006.01) , ***A61K 31/202*** (2006.01) , ***A61K 31/381*** (2006.01) , ***A61K 31/42*** (2006.01) , ***A61K 31/7105*** (2006.01) (more IPCs on the last page)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: ***A61K 31/575*** (2006.01) , ***A61K 31/122*** (2006.01) , ***A61K 31/202*** (2006.01) , ***A61K 31/381*** (2006.01) , ***A61K 31/42*** (2006.01) , ***A61K 31/7105*** (2006.01)) (more IPCs on the last page)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields
A61K

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms
Biosis, CAplus, Medline, WEST, Delphion, Canadian Patent Database, GenomeQuest (SEQ ID NO:1, all databases)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant	Relevant to claim No.
X	WO 2005/115358, (YALE UNIVERSITY), 04-05-2005 see page 6, lines 1-3 and 18; page 13 lines 17-23; page 20, lines 21-24	2, 3, 16-27, 31, 32, 45-57
X	WO 2007/013852, (AGENCY FOR SCIENCE, TECHNOLOGY & RESEARCH), 27-07-2005 Page 5, lines 29, 30; page 6, lines 1-3, 17, 22	2, 3, 16-27, 31, 32, 45-57
A	MOODLEY, et al., "Fibroblasts Isolated from Normal Lungs and Those with Idiopathic Pulmonary Fibrosis Differ in Interleukin-6/gp130-Mediated Cell Signaling and Proliferation", *Am. J. Pathol.*, **2003**, *163*(1), p345-354. See whole article	2, 3, 16-27, 31, 32, 45-57

[ ] Further documents are listed in the continuation of Box

[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
27 January 2009 (27-01-2009)	17 March 2009 (17-03-2009)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Karol Gajewski 819- 934-6734

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2008/001971

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

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

1. [X] Claim Nos. : 1-27
because they relate to subject matter not required to be searched by this Authority, namely :

Claims 1-27 is directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search. However, this Authority has carried out a search based on the alleged effect or purpose/use of the product defined in claim 57.

2. [X] Claim Nos. : 1, 28-30, 45-58 (in full) 2, 16-27, 31 (in part)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

These claims use vague and imprecise terms (i.e. functional terms) to define the claimed subject matter making a substantive search impossible. The search has been carried out based on subject matter supported by examples in the description.

3. [ ] Claim Nos. :
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. : 2, 16-27, 31, 45-57 (in part) 3, 32 (in full)

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

Continuation of Box No. III

Group 1: Claims 2, 16-27, 31, 45-57 (in part) 3, 32 (in full) are directed to the use of antisense RNA to treat an ILD,
Group 2: Claims 2, 16-27, 31, 45-57 (in part) 4, 33 (in full) are directed to the use of STA-21 (anthraquinone derivative) to treat an ILD,
Group 3: Claims 2, 16-27, 31, 45-57 (in part) 5, 34 (in full) are directed to the use of PIAS3 (Protein Inhibitor of Activated STAT3) to treat an ILD,
Group 4: Claims 2, 16-27, 31, 45-57 (in part) 6, 35 (in full) are directed to the use of LXA4 (C22 long chain acid) to treat an ILD,
Group 5: Claims 2, 16-27, 31, 45-57 (in part) 7, 36 (in full) are directed to the use of LY294002 (phenylchronenone) to treat an ILD,
Group 6: Claims 2, 16-27, 31, 45-57 (in part) 8, 37 (in full) are directed to the use of benzothiophene to treat an ILD,
Group 7: Claims 2, 16-27, 31, 45-57 (in part) 9-14, 38-43 (in full) are directed to the use of steroids to treat an ILD,
Group 8: Claims 2, 16-27, 31, 45-57 (in part) 15, 44 (in full) are directed to the use of Leflunomide to treat an ILD.
Group 9: Claim 58 is directed to a screening assay

Continuation of Page 4, Box A

A61K 38/17 (2006.01) , ***A61K 45/00*** (2006.01) , ***A61K 47/48*** (2006.01) , ***A61K 48/00*** (2006.01) ,
A61K 9/72 (2006.01) , ***A61P 11/00*** (2006.01) , ***C12Q 1/02*** (2006.01) , *A61K 9/00* (2006.01)

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO 2005115358A2	08-12-2005	US 2005265927A1	01-12-2005
		US 2008318895A1	25-12-2008
		WO 2005115358A3	09-03-2006
WO 2007013852A1	01-02-2007	EP 1915162A1	30-04-2008