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(54) Title: USING FATTY ACID SYNTHASE INHIBITORS TO TREAT FIBROSIS

(57) Abstract: This document relates to materials and methods for treating fibrosis (e.g., pulmonary fibrosis). For example, this document provides methods for using one or more fatty acid synthase (FASN) inhibitors to treat a mammal having fibrosis (e.g., pulmonary fibrosis), and/or a complication associated with fibrosis (e.g., hypoxia caused by pulmonary fibrosis).



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## USING FATTY ACID SYNTHASE INHIBITORS TO TREAT FIBROSIS

### Cross-Reference to Related Applications

This application claims the benefit of U.S. Patent Application Serial No. 62/311,615, filed on March 22, 2016. The disclosure of the foregoing application is hereby incorporated  
5 by reference in their entirety.

### Background

#### 1. Technical Field

This document relates to materials and methods for treating fibrosis (*e.g.*, pulmonary fibrosis). For example, this document provides methods for using one or more fatty acid  
10 synthase (FASN) inhibitors to treat a mammal having fibrosis (*e.g.*, pulmonary fibrosis), and/or a complication associated with fibrosis (*e.g.*, hypoxia caused by pulmonary fibrosis).

#### 2. Background Information

Fibrosis is the formation of excess fibrous connective tissue in an organ or tissue in a reparative or reactive process. Fibrosis can damage the architecture and function of the  
15 underlying organ or tissue. For example, pulmonary fibrosis occurs when lung tissue becomes damaged and scarred, making it difficult for your lungs to work properly.

Pulmonary fibrosis affects 200,000 people in the US, with an estimated 48,000 new cases are diagnosed each year (National Institutes of Health web page ([www.nhlbi.nih.gov](http://www.nhlbi.nih.gov)), 2010). Pulmonary fibrosis has a median survival rate of just two to three years, and more  
20 than 2/3 of patients will die within five years. There is no known cause and no cure for pulmonary fibrosis.

### Summary

This document provides materials and methods for treating fibrosis (*e.g.*, pulmonary fibrosis), and/or a complication associated with fibrosis (*e.g.*, hypoxia caused by pulmonary  
25 fibrosis). For example, this document provides methods and materials for administering one

or more FASN inhibitors to a mammal having fibrosis (*e.g.*, pulmonary fibrosis) under conditions wherein the severity of fibrosis is reduced. A FASN inhibitor can be an inhibitor of FASN polypeptide expression or an inhibitor of FASN polypeptide activity.

As demonstrated herein, FASN inhibitors can be effective to treat fibrosis and/or a complication associated with fibrosis. In some cases, one or more FASN inhibitors can be used to reduce the symptoms of fibrosis and/or a complication associated with fibrosis. In some cases, one or more FASN inhibitors can be used to decrease expression of profibrotic genes, to improve peripheral blood oxygenation, and/or to increase lung hydroxyproline content.

In general, one aspect of this document features a method for treating fibrosis in a mammal. The method includes, or consists essentially of, administering an inhibitor of FASN to a mammal identified said mammal as having fibrosis, wherein a symptom of said fibrosis is reduced. The mammal can be a human. The inhibitor can be C75. The inhibitor can be a FASN-targeting siRNA. The fibrosis can be pulmonary fibrosis (*e.g.*, idiopathic pulmonary fibrosis).

In another aspect, this document features a method for treating a complication associated with fibrosis in a mammal. The method includes, or consists essentially of, administering an inhibitor of fatty acid synthase activity to a mammal identified said mammal as having a complication associated fibrosis, wherein a symptom of said complication is reduced. The mammal can be a human. The inhibitor can be C75. The inhibitor can be a FASN-targeting siRNA. The fibrosis can be pulmonary fibrosis (*e.g.*, idiopathic pulmonary fibrosis). The complication associated with fibrosis can be hypoxia.

In another aspect, this document features a method for increasing polypeptide expression in cells. The method includes, or consists essentially of, contacting the cells with an inhibitor of fatty acid synthase activity, wherein expression of one or more profibrotic genes is decreased. The cells can be human cells. The cells can be lung cells. The inhibitor can be C75. The inhibitor can be a FASN-targeting siRNA. The one or more profibrotic genes can be selected from profibrotic genes collagen I, collagen IV, fibronectin, connective tissue growth factor, and alpha smooth muscle actin.

In another aspect, this document features a method for improving peripheral blood oxygenation in a mammal. The method includes, or consists essentially of, administering an inhibitor of FASN to a mammal identified a mammal, wherein peripheral blood oxygenation is increased in the mammal. The mammal can be a human. The inhibitor can be C75. The  
 5 inhibitor can be a FASN-targeting siRNA.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Methods and materials are described herein for use in the present disclosure; other, suitable methods and materials known in the art can also be used. The materials, methods,  
 10 and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

The details of one or more embodiments of the invention are set forth in the  
 15 accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

### Description of the Drawings

Figures 1A – 1H show FASN expression in mouse and human lung tissue. Figures 1A – 1C are histological sections of non-fibrotic (Saline:Saline (SS), A-C top), fibrotic (Bleomycin:Saline (BS), A-C middle), and fibrosis dual-treated with Imatinib and Lapatinib (Bleomycin:Imat + Lap (BIL) A-C bottom; which was previously shown to decrease pulmonary fibrosis, Andrianifahanana et al., 2013 *FASEB J* 27(11):4444-54) mouse lungs stained with Masson's Trichrome (A), FASN antibodies co-stained with Hematoxylin (B), or color deconvoluted images (C). Figure 1D is a graph of a quantification of FASN expression  
 20 of non-fibrotic (SS), fibrotic (BS), and dual-treated (BIL) mouse lung tissues; SS to BS \*p-value = 0.021, and BS to BIL \*p-value = 0.025. Figures 1E – 1F are histological sections of normal (E-G top), and fibrotic (E-G bottom) human lungs stained with Masson's Trichrome (E), FASN antibodies co-stained with Hematoxylin (F; arrows indicate normal fibroblasts

(top), or fibrotic foci observed in IPF (bottom)), or color deconvoluted images (G). Figure 1H is a graph of a quantification of FASN expression in human lung; \*p-value = 0.012.

Figures 2A – 2D show that TGF $\beta$  regulates FASN expression in murine and human fibroblasts. Figure 2A is a Western blot for FASN or actin (as a loading control) at the indicated times in the absence (-) or presence (+) of TGF $\beta$ . Figure 2B is a graph of the amount of FASN mRNA at the indicated times in the absence (vehicle) or presence of TGF $\beta$ . Figure 2C is a graph of relative luciferase activity in AKR-2B cultures transiently transfected with wild-type FASN (FASN-luc) or mutant FASN (FASN-Mut-luc) luciferase constructs; \*\*p-value < 0.01. Figure 2D is a Western blot for FASN or GAPDH (as a loading control) in murine (AKR-2B and Swiss 3T3) and human (MRC5) lung fibroblast lines or primary human lung fibroblasts in the absence (-) or presence (+) of TGF $\beta$ .

Figures 3A – 3E show FASN induction by TGF $\beta$  is independent of pSmad2/3 and mediated via mTORC1 signaling. Figure 3A is a Western blot for FASN, pSmad2, pSmad3, connective tissue growth factor (CTGF), and GAPDH (loading control) in AKR-2B cells transfected with either a nontargeting (NT) control siRNA or with Smad2/3-targeting siRNA in the absence (-) or presence (+) of TGF $\beta$ . Figure 3B is a Western blot for FASN, pSmad2, pSmad3, CTGF, and GAPDH (loading control) in MRC5 cells transfected with either a nontargeting (NT) control siRNA or with Smad2/3-targeting siRNA in the absence (-) or presence (+) of 10 ng/ml TGF $\beta$ . Figure 3C is a Western blot for FASN, pS6K, S6K, pErk, pSmad3, and GAPDH (loading control) in AKR-2B cells stimulated in the absence (-) or presence (+) of TGF $\beta$  plus LY294002, U0126, MK2206, Rapamycin, or Torin. Figure 3D is a Western blot for FASN, pS6K, S6K, pErk, pSmad3, and GAPDH (loading control) in MRC5 cells stimulated in the absence (-) or presence (+) of TGF $\beta$  plus LY294002, U0126, MK2206, Rapamycin, or Torin. Figure 3E is a Western blot for FASN, Rictor, Raptor, mTOR, and GAPDH (loading control) in AKR-2B cells cultured in the absence (-) or presence (+) of non-targeting short hairpin RNAs (shNT) as a control, or shRNAs targeting mTOR, Raptor, or Rictor clones.

Figures 4A – 4C show that FASN activity is required for profibrotic TGF $\beta$  signaling. Figure 4A is a Western blot for FASN, Collagen I (Col I), Collagen IV (Col IV), Fibronectin (FN), connective tissue growth factor (CTGF), pSmad3, and GAPDH (loading control) in

murine AKR-2B or human lung MRC5 fibroblasts treated with vehicle (-) or C75 in the presence (+) or absence (-) of TGF $\beta$ . Figure 4B is a graph of soft agar colony formation by AKR-2B cells in the presence or absence of TGF $\beta$  (10 ng/ml) and the indicated C75 concentration; \*p-value < 0.05, \*\*p-value < 0.01. Figure 4C shows scratch assays performed  
5 on AKR-2B or MRC5 cells in the absence (Con) or presence of TGF $\beta$  and C75; red bands indicate the leading edge following 24 hours in the absence (Con) or presence of TGF $\beta$  and C75.

Figures 5A – 5C shows that profibrotic TGF $\beta$  signaling is dependent upon the induction of fatty acid synthase. Figure 5A is a Western blot for FASN, Collagen I (Col I),  
10 Collagen IV (Col IV), Fibronectin (FN), connective tissue growth factor (CTGF), pSmad3, and GAPDH (loading control) in AKR-2B or MRC5 cells transfected with either a nontargeting (NT) control siRNA or with FASN-targeting siRNA in the absence (-) or presence (+) of TGF $\beta$ . Figure 5B is a graph of soft agar colony formation by AKR-2B cells transfected with either a nontargeting (NT) control siRNA or with FASN-targeting siRNA in  
15 the absence (-) or presence (+) of TGF $\beta$ ; \*\*p-value < 0.01. Figure 5C shows scratch assays performed on AKR-2B or MRC5 cells transfected with either a nontargeting (NT) control siRNA or with FASN-targeting siRNA in the absence (-) or presence (+) of TGF $\beta$ ; red bands indicate the leading edge following 24 hr in the absence (-) or presence (+) of TGF $\beta$ .

Figures 6A – 6B contain graphs showing inhibition of FASN does not inhibit in vitro  
20 cell proliferation. Figure 6A shows optical densities (at a wavelength of 570 nm; OD570) of AKR-2B cells grown in 0.1% FBS or 10% FBS, and MRC5 cells grown in FBS free or 10% FBS, and treated with vehicle (0.1% DMSO), or C75 (3  $\mu$ M). Figure 6B shows cell counts for AKR-2B cells and MRC5 cells grown in 0.1% FBS or 10% FBS, and MRC5 cells grown in FBS free or 10% FBS, and treated with vehicle (0.1% DMSO), or C75 (3  $\mu$ M).

Figures 7A – 7B contain graphs showing FASN inhibition has no effect on murine  
25 liver enzymes or inflammatory cell recruitment. Figure 7A shows serum levels (U/L, units per liter) of alkaline phosphatase (ALP), alanine aminotransferase (ALT), and albumin (AST) in mice challenged with bleomycin (BLM) or saline, and treated with vehicle or C75. Figure 7B shows quantification of lymphocytes, monocytes, and neutrophils in mice  
30 challenged with bleomycin (BLM) or saline, and treated with vehicle or C75.

Figure 8 includes graphs showing change in expression of fibronectin (FN), connective tissue growth factor (CTGF), and Collagen I (Col I) in murine lung tissue harvested on day 28 from mice challenged with bleomycin (+) or vehicle (-) and treated every 4th day with vehicle (-) or the indicated concentration (mg/kg) of C75 beginning 14 days following initial bleomycin insult; \*p-value < 0.05, \*\*p-value < 0.01.

Figure 9 is a graph showing oxygen saturation levels in mice challenged with bleomycin (BLM) or saline for 25 days and treated once every 4th day with vehicle or the indicated concentration (mg/kg) of C75 beginning 14 days after initial BLM insult; \*p-value < 0.05, \*\*p-value < 0.01.

Figures 10A – 10C contain graphs showing FASN inhibition stabilizes lung function in adenovirus-TGF $\beta$  model of pulmonary fibrosis. Figure 10A is a graph of peripheral blood oxygen determined on days 21 (d21) and 35 (d35) in mice infected adenovirus particles expressing GFP (control) or TGF $\beta$ 1, and treated with either vehicle or C75. Figure 10B is a graph showing lung hydroxyproline content in mice infected adenovirus particles expressing GFP (control) or TGF $\beta$ 1, and treated with either vehicle (-) or C75 (+). Figure 10C is a graph of qPCR expression of alpha smooth muscle actin ( $\alpha$ -SMA), collagen I $\alpha$ 1 (Col I $\alpha$ 1), and connective tissue growth factor (CTGF). \*P<0.05, \*\*P<0.01.

### Detailed Description

This document provides methods and materials for treating fibrosis. For example, this document provides methods and materials for using FASN inhibitors to treat fibrosis (*e.g.*, pulmonary fibrosis) and/or a complication associated with fibrosis (*e.g.*, hypoxia caused by pulmonary fibrosis). In some cases, one or more FASN inhibitors can be used to reduce the symptoms of fibrosis. In some cases, one or more FASN inhibitors can be used to decrease expression of profibrotic genes and/or to improve peripheral blood oxygenation.

When treating fibrosis as described herein, the fibrosis can be in any tissue. Fibrosis can occur in many tissues within the body including, without limitation, lungs (pulmonary fibrosis; *e.g.*, cystic fibrosis and/or idiopathic pulmonary fibrosis), liver (cirrhosis), heart (atrial fibrosis, endomyocardial fibrosis, old myocardial infarction), brain (glial scar), kidney (renal fibrosis; end stage renal diseases), ovary (ovarian cancer induced intestinal or

peritoneal adhesions), and pancreas (pancreatitis). Other types of fibrosis include arthrofibrosis (knee, shoulder, other joints), Crohn's Disease (intestine), Dupuytren's contracture (hands, fingers), keloid (skin), mediastinal fibrosis (soft tissue of the mediastinum), myelofibrosis (bone marrow), Peyronie's disease (penis), nephrogenic  
5 systemic fibrosis (skin), progressive massive fibrosis (lungs), retroperitoneal fibrosis (soft tissue of the retroperitoneum), and scleroderma/systemic sclerosis (skin, lungs). In some embodiments, the fibrosis treated as described herein can be pulmonary fibrosis, such as idiopathic pulmonary fibrosis.

When treating a complication associated with fibrosis as described herein, the  
10 complication associated with fibrosis can be hypoxia caused by pulmonary fibrosis. Other complications associated with fibrosis include, without limitation, pain and organ failure.

In some cases, the materials and methods provided herein can be used to reduce the symptoms of fibrosis. Symptoms of pulmonary fibrosis include, without limitation, reduced shortness of breath, coughing, and diminished exercise tolerance.

15 In some cases, the materials and methods provided herein can be used to improve peripheral blood oxygenation.

In some cases, the materials and methods provided herein can be used to increase lung hydroxyproline content.

In some cases, the materials and methods provided herein can be used to decrease  
20 expression of profibrotic genes (*e.g.*, Collagen I (Col I such as Col I $\alpha$ 1), Collagen IV (Col IV), Fibronectin (FN), connective tissue growth factor (CTGF), and alpha smooth muscle actin ( $\alpha$ -SMA)). Methods for decreasing expression of profibrotic genes in cells can include contacting the cells with one or more FASN inhibitors. Cells can be *in vivo* or *in vitro*. Cells can be from any appropriate sources (*e.g.*, mammalian cells such as human cells). In  
25 addition, the cells can be any type of cell including, without limitation, lung, liver, heart, neurons, osteoclasts, osteoblasts, chondrocytes, intestinal (*e.g.*, intestinal epithelial), and/or bone marrow (*e.g.*, hemopoietic or stromal). One or more FASN inhibitors can be contacted with the cells by any appropriate method. For example, in humans, a composition containing one or more FASN inhibitor described herein can be used to decrease expression of a human  
30 Col I polypeptide, a human Col IV polypeptide, a human FN polypeptide, a human CTGF

polypeptide, or a combination thereof. In some cases, a human Col I polypeptide can have an amino acid sequence set forth in, for example, National Center for Biotechnology Information (NCBI) Accession No: P02452 (see, *e.g.*, GI No. 296439504). In some cases, a human Col IV polypeptide can have an amino acid sequence set forth in, for example, NCBI  
5 Accession Nos: NP\_001836.3 (see, *e.g.*, GI No. 734520330), NP\_001290039.1 (see, *e.g.*, GI No. 734520332), NP\_001274687.1 (see, *e.g.*, GI No. 567757600), NP\_001274688.1 (see, *e.g.*, GI No. 567757602), NP\_001274689.1 (see, *e.g.*, GI No. 567757604), NP\_001838.2 (see, *e.g.*, GI No. 148536823), and NP\_001274687.1 (see, *e.g.*, GI No. 567757600). In some cases, a human FN polypeptide can have an amino acid sequence set forth in, for example,  
10 NCBI Accession No: P02751.4 (see, *e.g.*, GI No. 300669710). In some cases, a human CTGF polypeptide can have an amino acid sequence set forth in, for example, NCBI Accession No: NP\_001892 (see, *e.g.*, GI No. 4503123).

Any type of mammal having fibrosis (or a complication associated with fibrosis) or at risk for developing fibrosis (or a complication associated with fibrosis) can be treated as  
15 described herein. For example, humans and other primates such as monkeys having fibrosis can be treated with one or more FASN inhibitors. In some cases, dogs, cats, horses, cows, pigs, sheep, rabbits, mice, and rats can be treated with one or more FASN inhibitors as described herein.

Any appropriate method can be used to identify a mammal having fibrosis (or a  
20 complication associated with fibrosis) or as being at risk for developing fibrosis (or a complication associated with fibrosis). For example, imaging techniques (*e.g.*, chest x-ray, computerized topography (CT) scan, echocardiogram), lung function test (*e.g.*, pulmonary function test, oximetry, exercise stress test), and/or tissue sample analysis (*e.g.*, bronchoscopy, bronchoalveolar lavage, surgical biopsy) can be used to identify a human or  
25 other mammal having fibrosis.

Once identified as having fibrosis (or a complication associated with fibrosis) or as being at risk for developing fibrosis (or a complication associated with fibrosis), the mammal can be administered or instructed to self-administer one or more FASN inhibitors (*e.g.*, a composition containing one or more FASN inhibitors that reduce FASN polypeptide  
30 expression and/or activity).

A FASN inhibitor can be an inhibitor of FASN polypeptide expression or an inhibitor of FASN polypeptide activity. Examples of compounds that reduce FASN polypeptide activity include, without limitation, C75, orlistat, epigallocatechin-3-gallate (EGCG), triclosan, GSK837149A, GSK2194069, JNJ-54302833, IPI-9119, and TVB-2640 (Jones et al., 2015 *Clin Cancer Res* 21(24):5434-38). Examples of compounds that reduce FASN polypeptide expression include, without limitation, nucleic acid molecules designed to induce RNA interference (*e.g.*, a small interfering RNA (siRNA) molecule or a short hairpin RNA (shRNA) molecule), antisense molecules, and miRNAs. Additional FASN inhibitors can be readily designed based upon the nucleic acid and/or polypeptide sequences of FASN.

Examples of a FASN nucleic acids include, without limitation, the human FASN sequence set forth in GenBank® Accession Nos. AY451392.1 (see, *e.g.*, GI No. 41584441), BC063242.1 (see, *e.g.*, GI No. 38648666), and NM\_004104.4 (see, *e.g.*, GI No. 41872630). Examples of FASN polypeptides include, without limitation, the human FASN polypeptide having the amino acid sequence set forth in GenBank® accession Nos: AAA73576.1 (see, *e.g.*, GI No. 915392), AAS09886.1 (see, *e.g.*, GI No. 41584442), AAH63242.1 (see, *e.g.*, GI No. 38648667), EAW89745.1 (see, *e.g.*, GI No. 119610151), NP\_004095.4 (see, *e.g.*, GI No. 41872631), and P49327.3 (see, *e.g.*, GI No. 269849686).

In some cases, one or more FASN inhibitors (*e.g.*, one, two, three, four, five, or more FASN inhibitors) can be administered to a mammal to treat fibrosis (*e.g.*, pulmonary fibrosis) and/or a complication associated with fibrosis (*e.g.*, hypoxia caused by pulmonary fibrosis). For example, two or more FASN inhibitors can be administered to a mammal (*e.g.*, a human with fibrosis) to treat fibrosis (*e.g.*, pulmonary fibrosis).

A composition including one or more FASN inhibitors can be administered to a mammal having fibrosis (*e.g.*, pulmonary fibrosis) and/or a complication associated with fibrosis as a combination therapy with one or more additional agents/therapies used to treat fibrosis. For example, a combination therapy used to treat a mammal having fibrosis can include administering to the mammal (*e.g.*, a human) a composition including one or more FASN inhibitors and one or more fibrosis treatments such as medication (*e.g.*, corticosteroid (*e.g.*, prednisone), methotrexate, cyclosporine, acetylcysteine, perfenidone, and/or nintedanib), oxygen therapy, pulmonary rehabilitation, and/or surgery (lung transplant).

In embodiments where one or more FASN inhibitors are used in combination with one or more additional agents used to treat fibrosis, the one or more additional agents can be administered at the same time or independently. For example, the composition including one or more FASN inhibitors can be administered first, and the one or more additional agents administered second, or vice versa. In embodiments where one or more FASN inhibitors are used in combination with one or more additional therapies used to treat fibrosis, the one or more additional therapies can be performed at the same time or independently of the administration of one or more FASN inhibitors. For example, the composition including one or more FASN inhibitors can be administered before, during, or after the one or more additional therapies are performed.

In some cases, one or more FASN inhibitors can be formulated into a pharmaceutically acceptable composition for administration to a mammal having fibrosis and/or a complication associated with fibrosis. For example, a therapeutically effective amount of a FASN inhibitor can be formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents. A pharmaceutical composition can be formulated for administration in solid or liquid form including, without limitation, sterile solutions, suspensions, sustained-release formulations, tablets, capsules, pills, powders, and granules.

Pharmaceutically acceptable carriers, fillers, and vehicles that may be used in a pharmaceutical composition described herein include, without limitation, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

A pharmaceutical composition containing one or more FASN inhibitors can be designed for oral, parenteral (including subcutaneous, intramuscular, intravenous, and intradermal), or inhaled administration. When being administered orally, a pharmaceutical

composition containing one or more FASN inhibitors can be in the form of a pill, tablet, or capsule. Compositions suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions that can contain anti-oxidants, buffers, bacteriostats, and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. Compositions for inhalation can be delivered using, for example, an inhaler, a nebulizer, and/or a dry powder inhaler. The formulations can be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in a freeze dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules, and tablets.

In some cases, a pharmaceutically acceptable composition including one or more FASN inhibitors can be administered locally or systemically. For example, a composition containing a FASN inhibitor can be administered systemically by an oral administration to or inhalation by a mammal (*e.g.*, a human).

Effective doses can vary depending on the severity of the fibrosis and/or complication associate with fibrosis, the route of administration, the age and general health condition of the subject, excipient usage, the possibility of co-usage with other therapeutic treatments such as use of other agents, and the judgment of the treating physician.

An effective amount of a composition containing one or more FASN inhibitors can be any amount that reduces the severity of a symptom of a condition being treated (*e.g.*, a fibrosis and/or a complication associated with fibrosis) without producing significant toxicity to the mammal. For example, an effective amount of a FASN inhibitor such as C75 can be from about 0.3 mg/kg to about 50 mg/kg (*e.g.*, from about 0.4 mg/kg to about 40 mg/kg, from about 0.5 mg/kg to about 30 mg/kg, from about 1 mg/kg to about 20 mg/kg, from about 2 mg/kg to about 15 mg/kg, or from about 3 mg/kg to about 10 mg/kg). The effective amount can remain constant or can be adjusted as a sliding scale or variable dose depending on the mammal's response to treatment. Various factors can influence the actual effective amount used for a particular application. For example, the frequency of administration, duration of treatment, use of multiple treatment agents, route of administration, and severity

of the condition (*e.g.*, a fibrosis and/or a complication associated with fibrosis) may require an increase or decrease in the actual effective amount administered.

The frequency of administration can be any frequency that reduces the severity of a symptom of a condition to be treated (*e.g.*, a fibrosis and/or a complication associated with fibrosis) without producing significant toxicity to the mammal. For example, the frequency of administration can be from about once a week to about three times a day, from about twice a month to about six times a day, or from about twice a week to about once a day. The frequency of administration can remain constant or can be variable during the duration of treatment. A course of treatment with a composition containing one or more FASN inhibitors can include rest periods. For example, a composition containing one or more FASN inhibitors can be administered daily over a two week period followed by a two week rest period, and such a regimen can be repeated multiple times. As with the effective amount, various factors can influence the actual frequency of administration used for a particular application. For example, the effective amount, duration of treatment, use of multiple treatment agents, route of administration, and severity of the condition (*e.g.*, a fibrosis and/or a complication associated with fibrosis) may require an increase or decrease in administration frequency.

An effective duration for administering a composition containing one or more FASN inhibitors can be any duration that reduces the severity of a symptom of the condition to be treated (*e.g.*, a fibrosis and/or a complication associated with fibrosis) without producing significant toxicity to the mammal. For example, the effective duration can vary from several days to several weeks, months, or years. In some cases, the effective duration for the treatment of a fibrosis and/or a complication associated with fibrosis can range in duration from about one month to about 10 years. Multiple factors can influence the actual effective duration used for a particular treatment. For example, an effective duration can vary with the frequency of administration, effective amount, use of multiple treatment agents, route of administration, and severity of the condition being treated.

In certain instances, a course of treatment and the severity of one or more symptoms related to the condition being treated (*e.g.*, a fibrosis and/or a complication associated with fibrosis) can be monitored. Any appropriate method can be used to determine whether or not

the severity of a symptom is reduced. For example, the severity of a symptom of fibrosis can be assessed using imaging techniques (*e.g.*, chest x-ray, CT scan, echocardiogram), lung function test (*e.g.*, pulmonary function test, oximetry, exercise stress test), and/or tissue sample analysis (*e.g.*, bronchoscopy, bronchoalveolar lavage, surgical biopsy) at different time points.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

## Examples

### Example 1: FASN Expression in mouse and human lung tissues

Mice were treated with bleomycin to induce lung fibrosis or treated with saline as a control. Bleomycin-treated animals were treated dual-treated with lapatinib (an ErbB inhibitor) and imatinib (a PDGF receptor and cAbl inhibitor) to decrease lung fibrosis and stabilize peripheral blood oxygenation. Lungs were harvested and histological sections were prepared. Mouse lungs were stained with Masson's Trichrome (Figure 1A), or FASN antibodies co-stained with hematoxylin (Figure 1B). Color deconvoluted images of mouse lungs stained with FASN (Figure 1C) indicate the area and intensity of FASN staining. Color-coded legend indicating intensity of FASN expression is provided.

Histological sections of normal and fibrotic lung tissues were stained with Masson's Trichrome (Figure 1E), or FASN antibodies co-stained with Hematoxylin (Figure 1F). Arrows indicate the magnified section showing fibroblasts found in normal human lung (Figures E-G top), or fibrotic foci observed in IPF (Figures E-G bottom). Color deconvoluted images are shown of FASN stained human lungs (Figure H). Color-coded legend indicating intensity of FASN expression is provided.

FASN expression was quantified in mouse and human lung tissues. FASN expression in mouse lung tissues was significantly greater in fibrosis versus normal (\*p-value = 0.021), or dual-treated lungs with imatinib + lapatinib (\*p-value = 0.025) (Figure 1D). Mean values of mouse tissues analyzed per condition are indicated within each bar (n=3) and error bars show the standard error (Figure 1D). FASN expression in human lung fibrotic foci

was significantly greater than fibroblasts in normal human lung (\*p-value = 0.012) (Figure 1H). Mean values of human cases are indicated within each bar (normal n=7; fibrotic n=12), and error bars show the standard error (Figure 1H).

These results show that FASN expression correlates with lung fibrosis.

#### 5                                    Example 2: TGF $\beta$ regulates FASN expression

Quiescent AKR-2B fibroblasts were stimulated in the absence or presence of TGF $\beta$  (10 ng/ml). Proteins and total RNA were obtained from treated and control cells. Protein samples were Western blotted for FASN or actin at the indicated times, and RNA samples (500 ng) were analyzed by RT-PCR using FASN primers. TGF $\beta$  stimulated FASN  
10    expression (Figure 2A). TGF $\beta$  increased FASN transcription (Figure 2B).

AKR-2B cultures were transiently transfected with wild-type FASN (FASN-luc) or mutant FASN promoter (FASN-Mut-luc) luciferase constructs and were treated with vehicle or TGF $\beta$  (10 ng/ml). Following 24 hours of treatment, normalized luciferase activity was determined. As shown in Figure 2C, wild-type FASN exhibited significantly more luciferase  
15    activity than mutant FASN (n=3; \*\*p-value < 0.01).

Murine (AKR-2B and Swiss 3T3) and human (MRC5) lung fibroblast cells and primary human lung fibroblasts were stimulated for 24 hours in the absence or presence of 10 ng/ml TGF $\beta$ . Proteins were obtained from treated and control cells. Protein samples were Western blotted for FASN or GAPDH. TGF $\beta$  induced FASN expression in murine  
20    fibroblasts, human lung fibroblast cell lines, and primary lung fibroblasts (Figure 2D).

These results show that TGF $\beta$  regulates FASN expression.

#### Example 3: FASN induction by TGF $\beta$

Murine fibroblasts (AKR-2B) and human lung fibroblasts (MRC5) were transfected with either a nontargeting control siRNA (60 nmol/L) or with Smad2/3-targeting siRNA (60  
25    nmol/L). After 72 hours of cultivation, the transient transfectants were stimulated in the absence or presence of 10 ng/ml TGF $\beta$  for 24 hours. Proteins were obtained from treated and control cells. Protein samples were Western blotted for FASN, pSmad2, pSmad3, and connective tissue growth factor (CTGF), and Western blotted for GAPDH as a loading

control. TGF $\beta$  induced FASN expression was independent of pSmad2/3 (Figures 3A and 3B).

Murine fibroblasts (AKR-2B) and human lung fibroblasts (MRC5) were stimulated in the absence or presence of TGF $\beta$  (10 ng/ml) plus a PI3K inhibitor (LY294002; 10  $\mu$ M), a MEK inhibitor (U0126; 10  $\mu$ M), an AKT inhibitor (MK2206; 0.3  $\mu$ M), a mTORC1 inhibitor (Rapamycin; 100 nM), or a mTORC1,2 inhibitor (Torin; 200 nM). Expression of pErk and pS6K/pS6K was determined at 1 and 6 hours post-stimulation while FASN, pSmad3, and GAPDH assessed at 24 hours. TGF $\beta$  induced FASN expression was abrogated in the presence of rapamycin or torin (Figures 3C and 3D).

Murine fibroblasts (AKR-2B) transfected with either non-targeting (NT) short hairpin (sh) RNAs or shRNAs targeting mTOR, Raptor, or Rictor were treated in the absence or presence of 10 ng/ml TGF $\beta$  for 24 hours. Proteins were obtained from treated and control cells, and protein samples were Western blotted for FASN, Rictor, Raptor, mTor, and GAPDH (Figure 3E).

These results show that FASN induction by TGF $\beta$  is independent of pSmad2/3 and mediated via mTORC1 signaling.

#### Example 4: FASN activity and profibrotic TGF $\beta$ signaling

Murine fibroblasts (AKR-2B) and human lung fibroblasts (MRC5) were treated for 24 hours with vehicle (0.025% DMSO) or C75 (FASN inhibitor, 3  $\mu$ M) in the presence or absence of TGF $\beta$  (10 ng/ml). Proteins were obtained from treated and control cells. Protein samples were Western blotted for FASN, Col I, Col IV, FN, CTGF, and pSmad3, and Western blotted for GAPDH as a loading control. TGF $\beta$  increased expression of FASN, Col I, Col IV, FN, CTGF, and pSmad3, but inhibition of FASN activity with C75 suppressed TGF $\beta$  induced Col I, Col IV, and FN expression (Figure 4A).

Murine fibroblasts (AKR-2B) were treated for 24 hours with vehicle (0.025% DMSO) or C75 (FASN inhibitor, 3  $\mu$ M) in the presence or absence of TGF $\beta$  (10 ng/ml), and 0, 0.3, 1, 3, or 10  $\mu$ M C75. Following 7 days growth, cells were subjected to soft agar colony formation assays. TGF $\beta$  increased colony formation, but inhibition of FASN activity with C75 suppressed TGF $\beta$  induced colony formation in a dose dependent manner (Figure 4B; \*p-

value < 0.05, \*\*p-value < 0.01). Data reflect the mean +/- standard deviation of triplicate wells from 3 experiments.

Murine fibroblasts (AKR-2B) and human lung fibroblasts (MRC5) were treated for 24 hours with vehicle (0.025% DMSO), TGFβ (10 ng/ml), or both TGFβ (10 ng/ml) and C75 (3 μM). Following 24 hours of treatment, culture plates were manually scratched. TGFβ increased cell migration, but inhibition of FASN activity with C75 suppressed TGFβ induced cell migration (Figure 4C). Data are representative of 3 separate experiments.

These results show that FASN activity is required for profibrotic TGFβ signaling.

#### Example 5: Profibrotic TGFβ signaling induction of fatty acid synthase

Murine fibroblasts (AKR-2B) and human lung fibroblasts (MRC5) were transfected with either a nontargeting control siRNA (60 nmol/L) or with FASN-targeting siRNA (60 nmol/L). After 72 hours of cultivation, the transient transfectants were stimulated in the absence or presence of 10 ng/ml TGFβ for 24 hours. Proteins were obtained from treated and control cells. Protein samples were Western blotted for FASN, Col I, Col IV, FN, CTGF, and pSmad3, and Western blotted for GAPDH as a loading control. TGFβ increased expression of FASN, Col I, Col IV, FN, CTGF, and pSmad3, but inhibition of FASN activity with FASN-targeting siRNA suppressed TGFβ induced Col I, Col IV, and FN expression (Figure 5A).

Murine fibroblasts (AKR-2B) were transfected with either a nontargeting control siRNA (60 nmol/L) or with FASN-targeting siRNA (60 nmol/L). After 72 hours of cultivation, the transient transfectants were stimulated in the absence or presence of 10 ng/ml TGFβ for 24 hours. Following 7 days growth, cells were subjected to soft agar colony formation assays. TGFβ increased colony formation, but inhibition of FASN activity with FASN-targeting siRNA suppressed TGFβ induced colony formation (Figure 5B; \*\*p-value < 0.01). Data reflect the mean standard deviation of triplicate wells from 3 experiments.

Murine (AKR-2B) lung fibroblasts were transfected with either a nontargeting control siRNA (60 nmol/L) or with FASN-targeting siRNA (60 nmol/L). After 72 hours of cultivation, the transient transfectants were stimulated in the absence or presence of 10 ng/ml TGFβ for 24 hours. Following 24 hours of treatment, culture plates were manually

scratched. TGF $\beta$  increased cell migration, but inhibition of FASN activity with FASN-targeting siRNA suppressed TGF $\beta$  induced cell migration (Figure 5C). Data are representative of 3 separate experiments.

These results show that profibrotic TGF $\beta$  signaling is dependent on FASN induction.

5                                    Example 6: Inhibition of FASN and cell proliferation

Murine fibroblasts (AKR-2B) and human lung fibroblasts (MRC5) were seeded at  $2.5 \times 10^3$  or  $1 \times 10^4$  cells/96 well plate, respectively. AKR-2B cells were seeded in medium containing 10% DMEM/FBS, and MRC5 cells were seeded in medium containing 10% EMEM/FBS. 24 hours after seeding, the medium was removed and replaced with DMEM or  
10 EMEM containing vehicle (0.1% DMSO), or C75 (3  $\mu$ M) either in 10%, 0.1%, or 0% FBS for 24 hours prior to MTT assay (Figure 6A). Absorbance was measured at 570 nm. Results represent mean  $\pm$  SEM from three independent experiments

Murine fibroblasts (AKR-2B) and human lung fibroblasts (MRC5) were seeded at  $1.0 \times 10^4$  cells/well in 24 well plates for 24 hours. AKR-2B cells were seeded in medium  
15 containing 10% DMEM/FBS, and MRC5 cells were seeded in medium containing 10% EMEM/FBS. 24 hours after seeding, the medium was removed and replaced with DMEM or EMEM containing vehicle (0.1% DMSO), or C75 (3  $\mu$ M) either in 10%, 0.1%, or 0% FBS for 24 hours prior to determining cell counts following an additional 24 hour and 48 hour incubation (Figure 6B). Results represent mean  $\pm$  SEM from three independent experiments.

20                                    These results show that inhibition of FASN does not inhibit *in vitro* cell proliferation.

Example 7: Inhibition FASN and liver enzymes or inflammatory cell recruitment

C57BL/6 mice were intratracheally treated with an equal volume of saline (control) or bleomycin (BLM; 0.075U). On day 14 all animals began treatment every 4<sup>th</sup> day with either vehicle (Methocel/saline) or 3.0 mg/kg of C75. Blood samples were obtained at days 0, 14,  
25 and 28 from the facial vein of unanesthetized animals and assessed for effect on liver enzymes. Serum levels (U/L, units per liter; g/dL, grams per deciliter) of alkaline phosphatase (ALP), alanine aminotransferase (ALT), and albumin were determined using a

Piccolo Xpress Chemistry Analyzer (Figure 7A). Data are presented as mean +/- SEM of n=5.

C57BL/6 mice were intratracheally treated with an equal volume of saline (control) or bleomycin (BLM; 0.075U). On day 14 all animals began treatment every 4<sup>th</sup> day with either vehicle (Methocel/saline) or 3.0 mg/kg of C75. Blood samples were obtained at days 0, 14, and 28 from the facial vein of unanesthetized animals and assessed for effect on inflammatory cells. Quantification of lymphocytes, monocytes, and neutrophils were measured using a VetScan HM5 Analyzer (Figure 7B). Data are presented as mean +/- SEM of n=5.

These results show that inhibition of FASN has no demonstrable effect on murine liver enzymes or inflammatory cell recruitment.

#### Example 8: Inhibition of FASN and lung remodeling

Mice were challenged with bleomycin or vehicle (methocel/0.1%DMSO), and every 4<sup>th</sup> day, beginning 14 days following initial bleomycin insult, were treated by intraperitoneal injection with vehicle, 0.03 mg/kg, 0.3 mg/kg, or 3 mg/kg C75. Murine lung tissue was harvested on day 28 and RNA was obtained. qPCR was performed using primers specific for FN, CTGF, and Col I.

Bleomycin-induced fibrotic conditions demonstrated increased FN, CTGF, and Col I expression, but inhibition of FASN activity with C75 reduced FN, CTGF, and Col I expression in a dose dependent manner (Figure 6; \*p-value < 0.05, \*\*p-value < 0.01). Data reflect mean standard deviation from lungs of n=5 (control, bleomycin, 0.03 mg/kg C75), 7 (0.3 mg/kg C75), or 8 (3 mg/kg C75) animals.

These results show that FASN inhibition prevents profibrotic gene expression in lungs and that lung remodeling is attenuated by inhibiting FASN activity.

#### Example 9: Inhibition of FASN and lung gas exchange

Mice were challenged with bleomycin or saline for 25 days, and every 4<sup>th</sup> day, beginning 14 days following initial bleomycin insult, were treated with vehicle

(methocel/0.1%DMSO) or with 0.03 mg/kg, 0.3 mg/kg, or 3 mg/kg C75. Oxygen saturation (SpO<sub>2</sub>) levels (determined on room air) were measured.

Bleomycin-induced fibrotic conditions caused reduced, time-dependent fluctuations in oxygen saturation, but inhibition of FASN activity with C75 stabilized lung gas exchange in a dose dependent manner (Figure 7; \*p-value < 0.05, \*\*p-value < 0.01). Error bars reflect standard deviation from n=5 (control, BLM, 0.03 mg/kg C75), 7 (0.3 mg/kg C75), or 8 (3 mg/kg C75) animals.

These results show that FASN inhibition maintains/improves peripheral blood oxygenation.

#### Example 10: Inhibition FASN and lung function

Mice were infected with  $1 \times 10^8$  pfu adenovirus particles expressing control (GFP) or active TGF $\beta$ 1 by tracheal instillation. On day 21 all animals began treatment every 4<sup>th</sup> day with either vehicle (Methocel/saline) or 3.0 mg/kg of C75. Peripheral blood oxygen determined on days 21 and 35 (Figure 10A). Data reflect mean +/- SEM of n=8 and n=15 for adenovirus-GFP and adenovirus-TGF $\beta$ , respectively. \*P<0.05.

Mice were infected with  $1 \times 10^8$  pfu adenovirus particles expressing control (GFP) or active TGF $\beta$ 1 by tracheal instillation. On day 21 all animals began treatment every 4<sup>th</sup> day with either vehicle (Methocel/saline) or 3.0 mg/kg of C75. Mice were sacrificed on day 39 and processed for lung hydroxyproline content (Figure 10B). Data reflect mean +/- SEM of n=8 and n=15 for adenovirus-GFP and adenovirus-TGF $\beta$ , respectively. \*\*P<0.01.

Mice were infected with  $1 \times 10^8$  pfu adenovirus particles expressing control (GFP) or active TGF $\beta$ 1 by tracheal instillation. On day 21 all animals began treatment every 4<sup>th</sup> day with either vehicle (Methocel/saline) or 3.0 mg/kg of C75. Mice were sacrificed on day 39 and processed for qPCR expression of connective tissue growth factor (CTGF), alpha smooth muscle actin ( $\alpha$ -SMA), and collagen I $\alpha$ 1 (Col I $\alpha$ 1). Data reflect mean +/- SEM of n=8 and n=15 for adenovirus-GFP and adenovirus-TGF $\beta$ , respectively. \*P<0.05, \*\*P<0.01.

These results show that inhibition of FASN stabilizes lung function in adenovirus-TGF $\beta$  model of pulmonary fibrosis.

Other Embodiments

It is to be understood that while the disclosure has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the disclosure, which is defined by the scope of the appended claims.

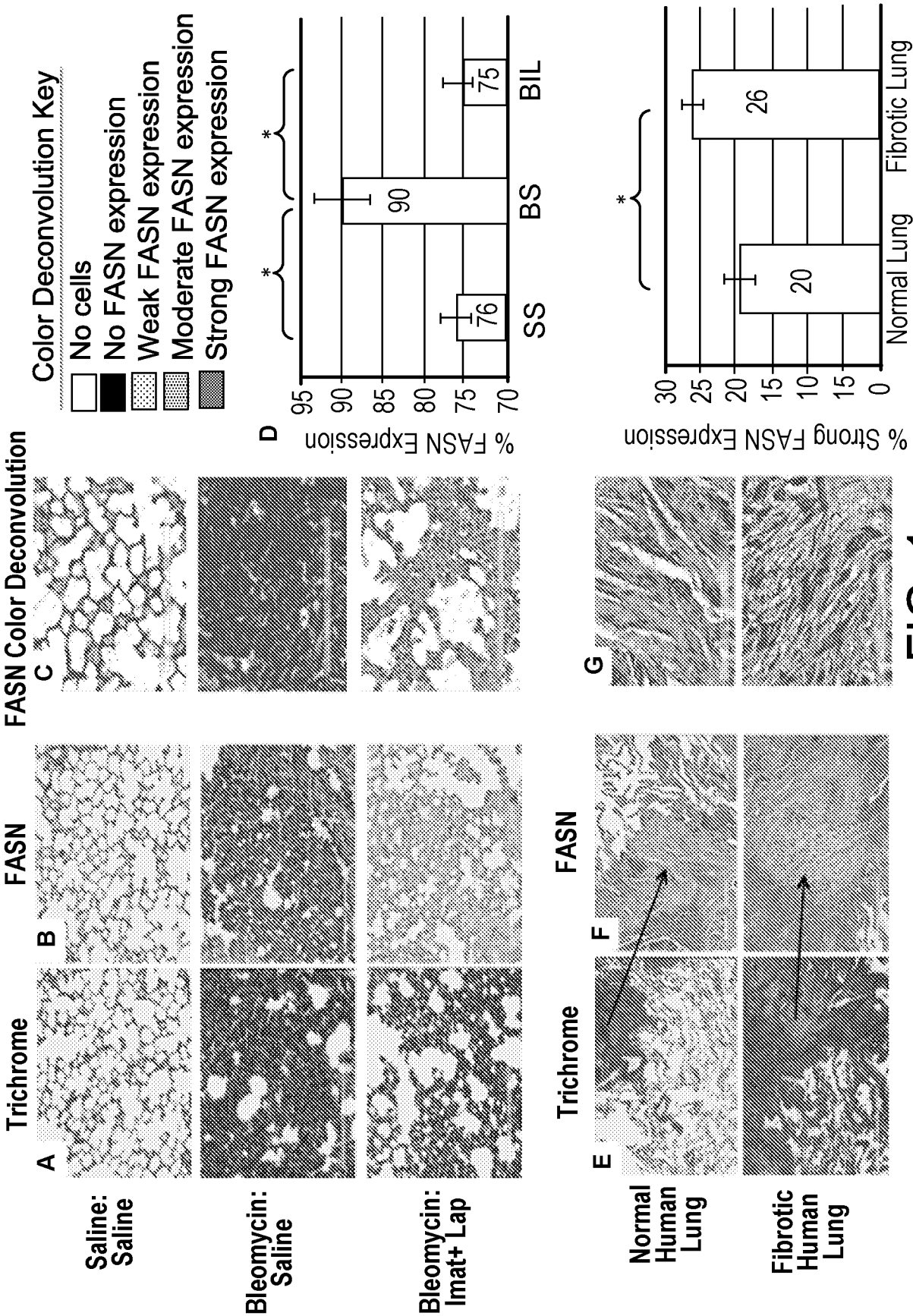
- 5 Other aspects, advantages, and modifications are within the scope of the following claims.

**WHAT IS CLAIMED IS:**

1. A method for treating fibrosis in a mammal, said method comprising:  
administering an inhibitor of fatty acid synthase activity to a mammal identified as having fibrosis;  
wherein a symptom of said fibrosis is reduced.
2. The method of claim 1, wherein said mammal is a human.
3. The method of any one of claims 1-2, wherein said inhibitor is C75.
4. The method of any one of claims 1-2, wherein said inhibitor is a fatty acid synthase targeting small interfering RNA.
5. The method of any one of claims 1-4, wherein said fibrosis is pulmonary fibrosis.
6. The method of claim 5, wherein said pulmonary fibrosis is idiopathic pulmonary fibrosis.
7. A method for treating a complication associated with fibrosis in a mammal, said method comprising:  
administering an inhibitor of fatty acid synthase activity to a mammal identified as having a complication associated fibrosis;  
wherein a symptom of said complication is reduced.
8. The method of claim 7, wherein said mammal is a human.
9. The method of any one of claims 7-8, wherein said inhibitor is C75.

10. The method of any one of claims 7-8, wherein said inhibitor is a fatty acid synthase targeting small interfering RNA.
11. The method of any one of claims 7-10, wherein said fibrosis is pulmonary fibrosis.
12. The method of claim 11, wherein said pulmonary fibrosis is idiopathic pulmonary fibrosis.
13. The method of claim 11, wherein said complication associated with fibrosis is hypoxia.
14. A method for increasing polypeptide expression in cells, the method comprising:  
contacting said cells with an inhibitor of fatty acid synthase activity;  
wherein expression of one or more profibrotic genes is decreased.
15. The method of claim 14, wherein said cells are human cells.
16. The method of any one of claims 14-15, wherein said cells are lung cells.
17. The method of any one of claims 14-16, wherein said inhibitor is C75.
18. The method of any one of claims 14-16, wherein said inhibitor is a fatty acid synthase targeting small interfering RNA.
19. The method of any one of claims 14-18, wherein said one or more profibrotic genes are selected from the group consisting of profibrotic genes collagen I, collagen IV, fibronectin, connective tissue growth factor, and alpha smooth muscle actin.

20. A method for improving peripheral blood oxygenation in a mammal, the method comprising:
- administering an inhibitor of fatty acid synthase activity to a mammal;
  - wherein peripheral blood oxygenation is increased in the mammal.
21. The method of claim 20, wherein said mammal is a human.
22. The method of any one of claims 20-21, wherein said inhibitor is C75.
23. The method of any one of claims 20-21, wherein said inhibitor is a fatty acid synthase targeting small interfering RNA.



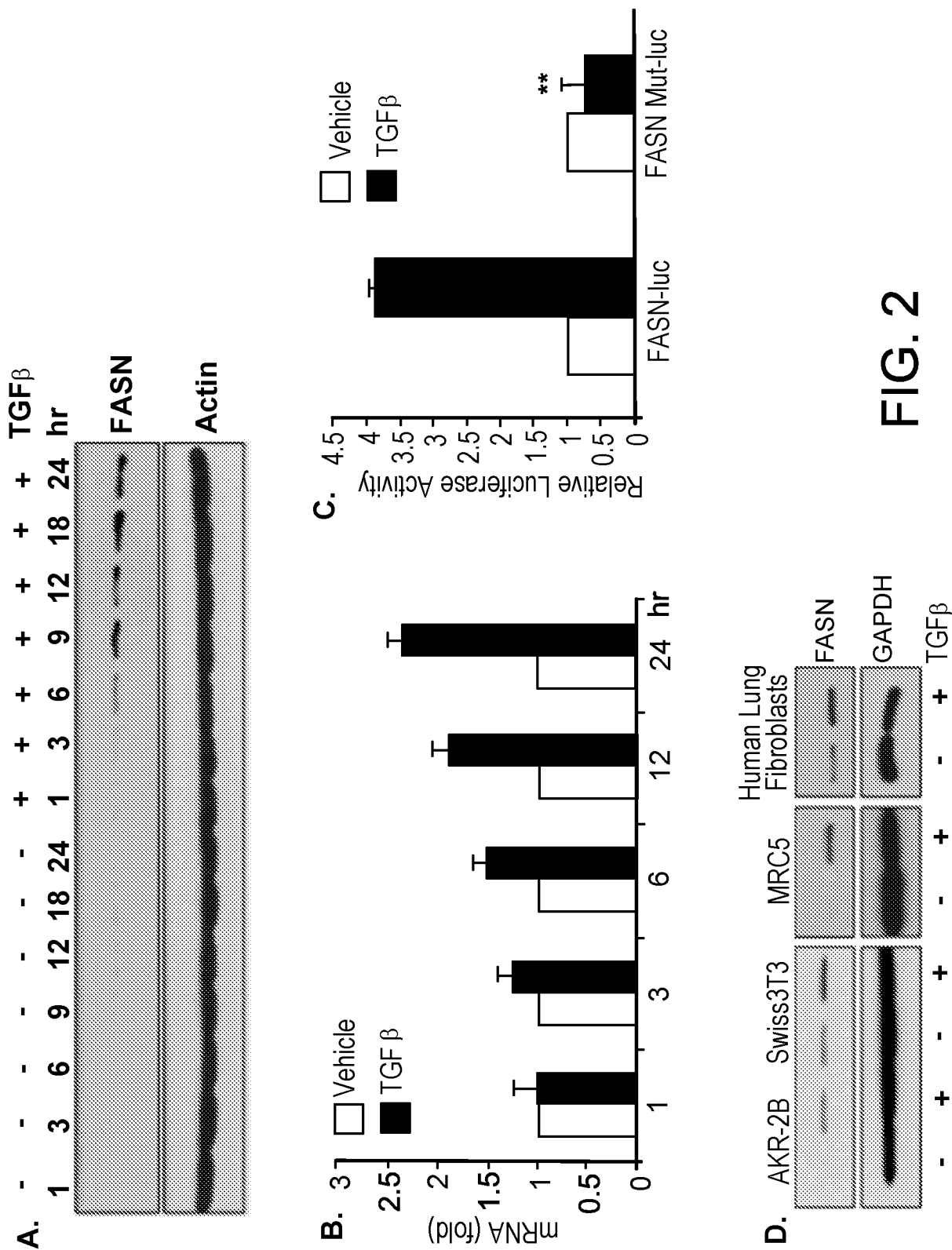


FIG. 2

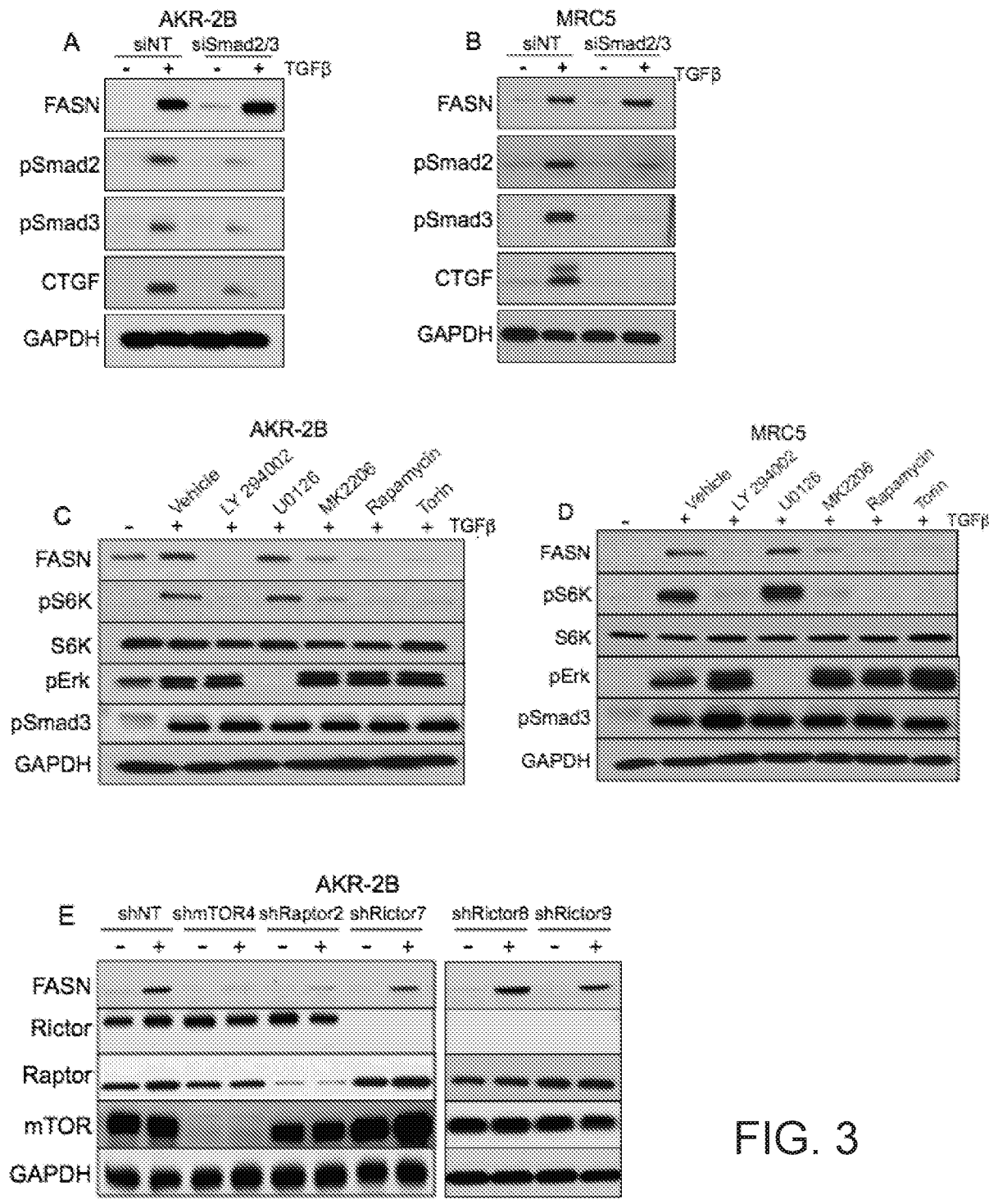


FIG. 3

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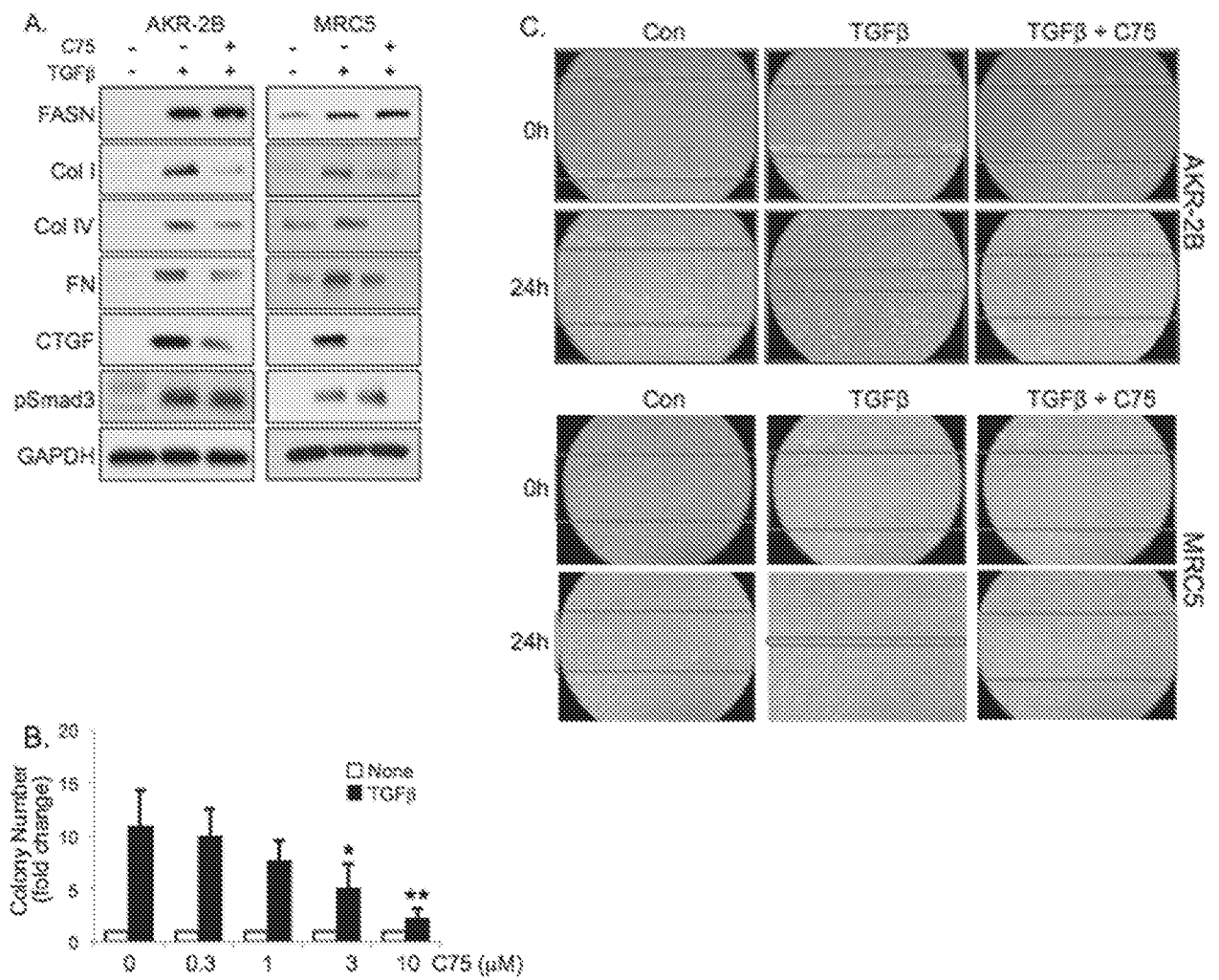


FIG. 4

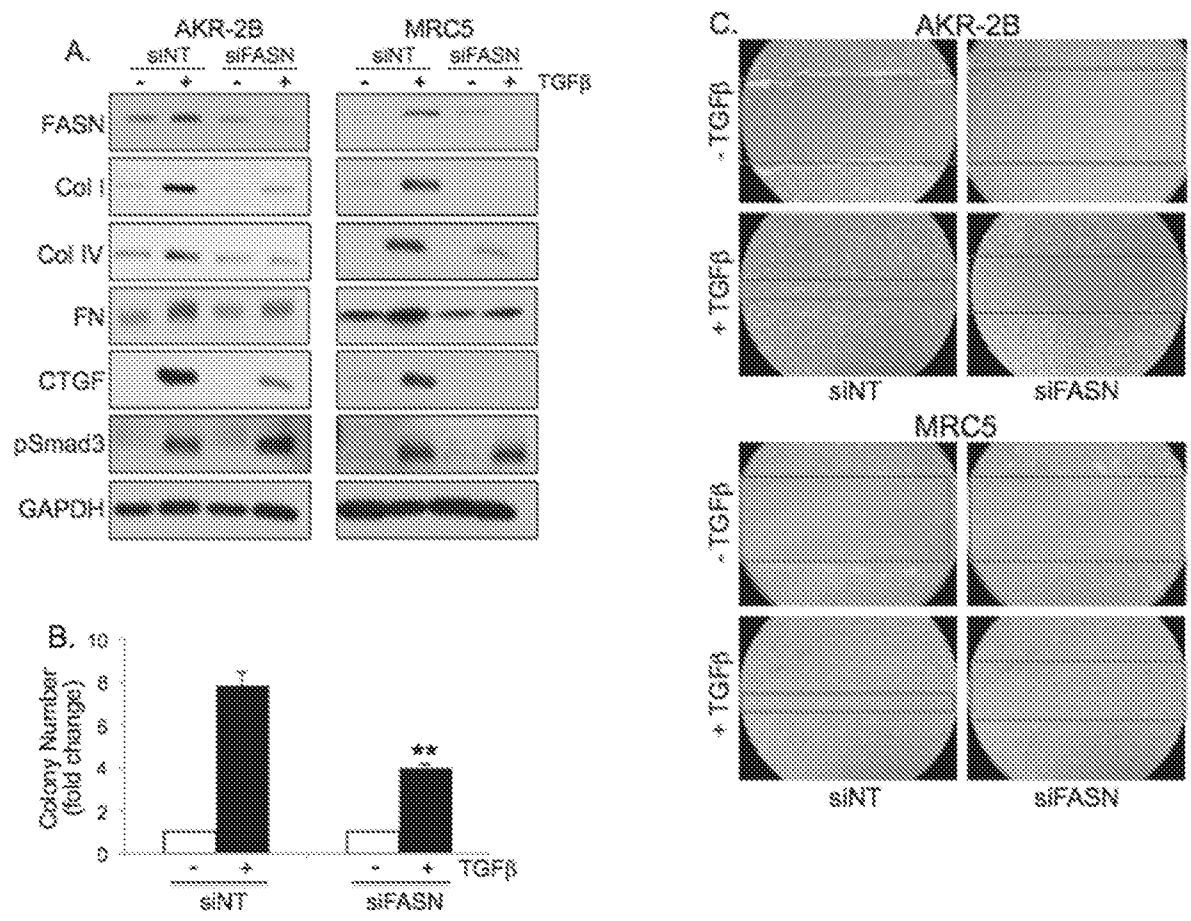


FIG. 5

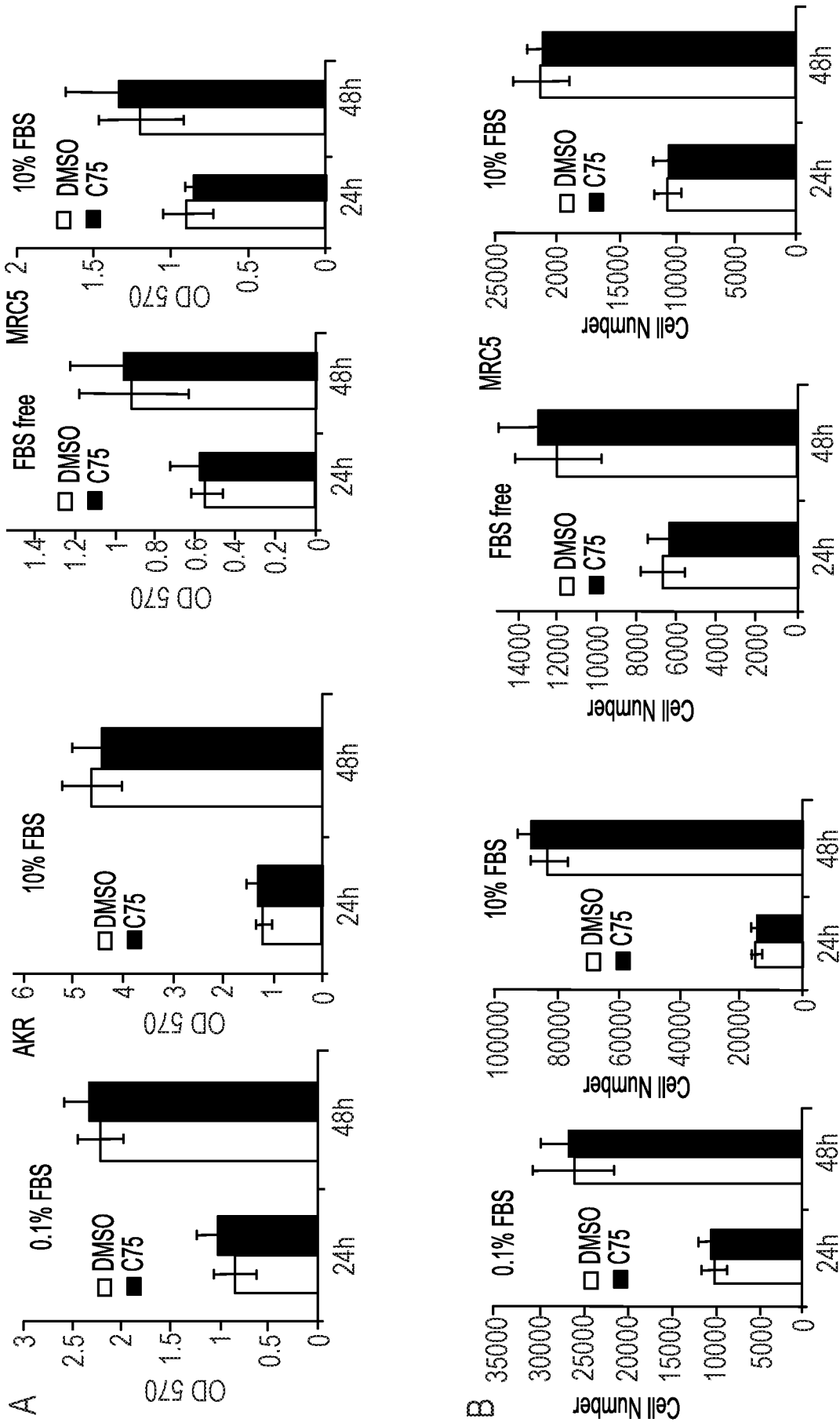
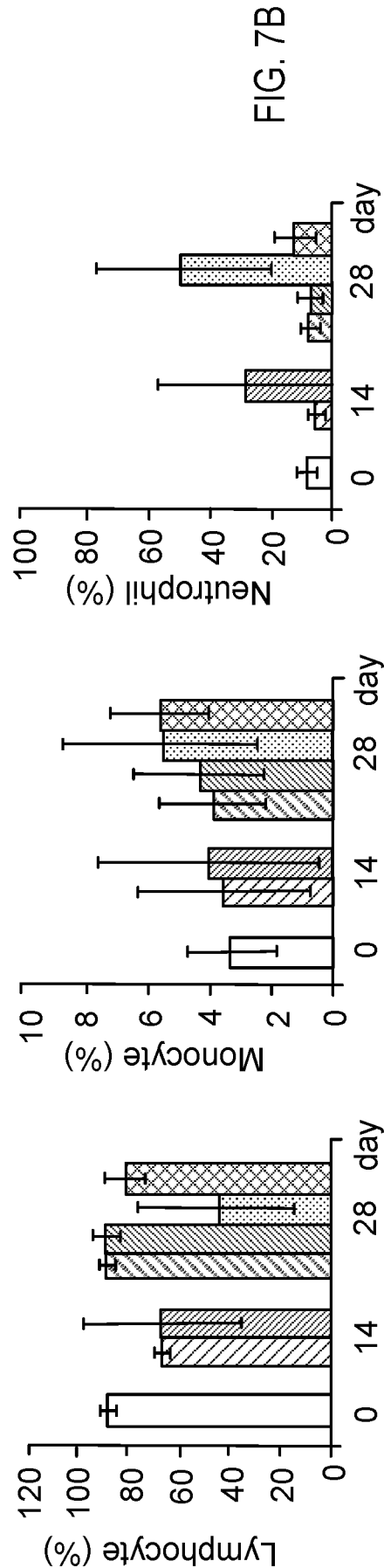
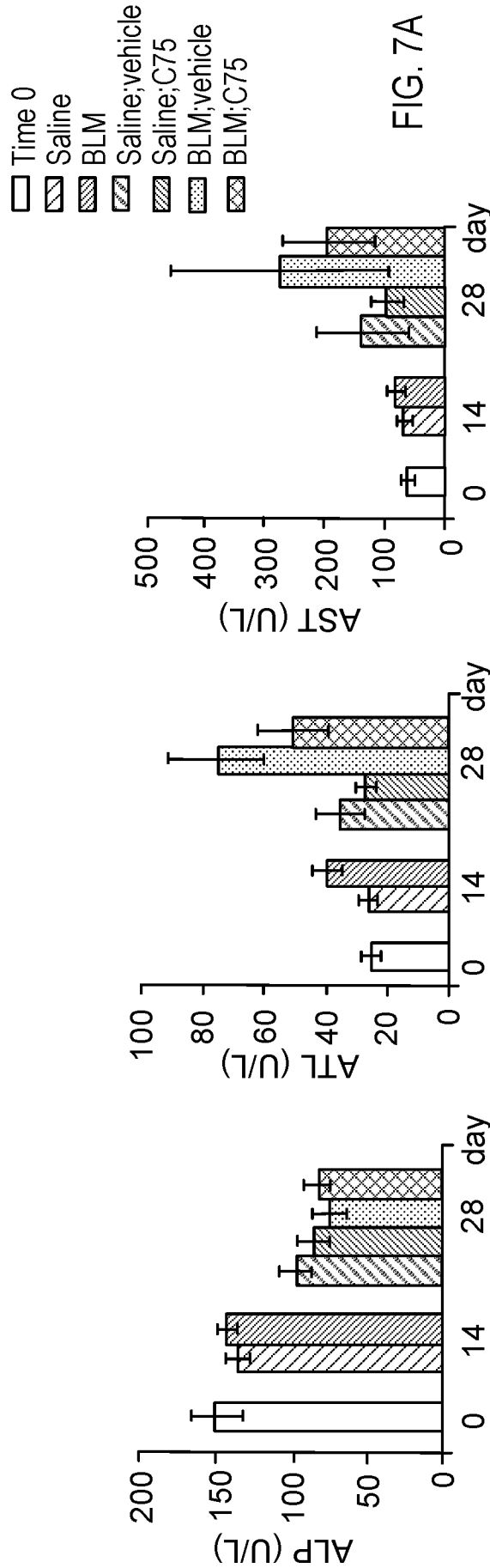


FIG. 6



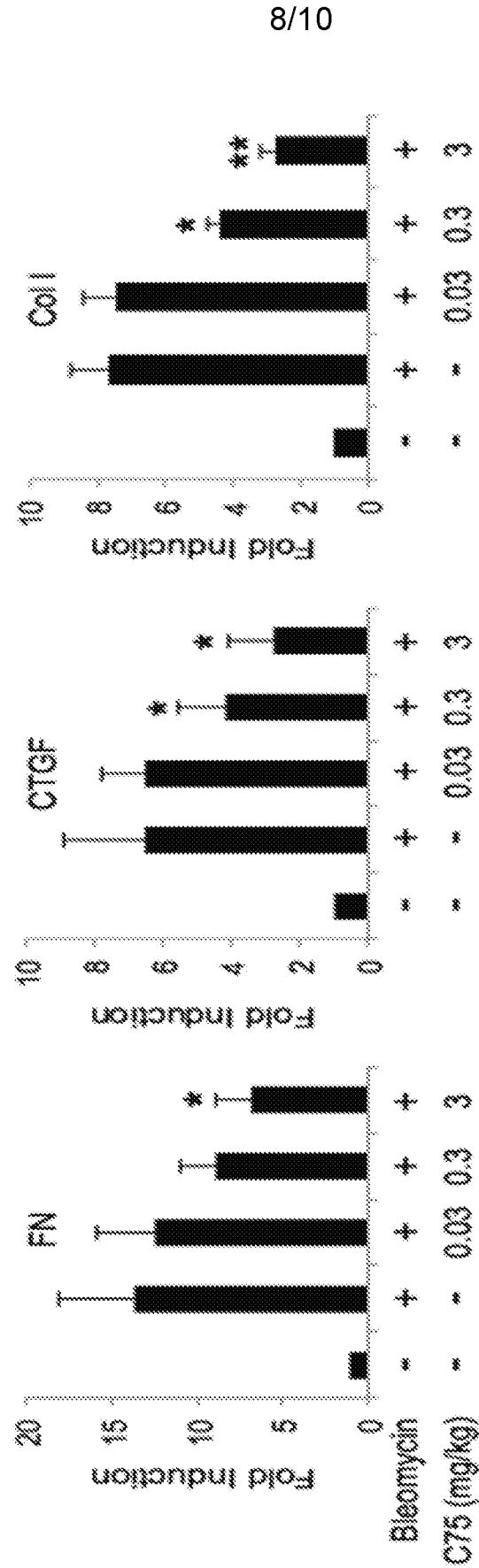


FIG. 8

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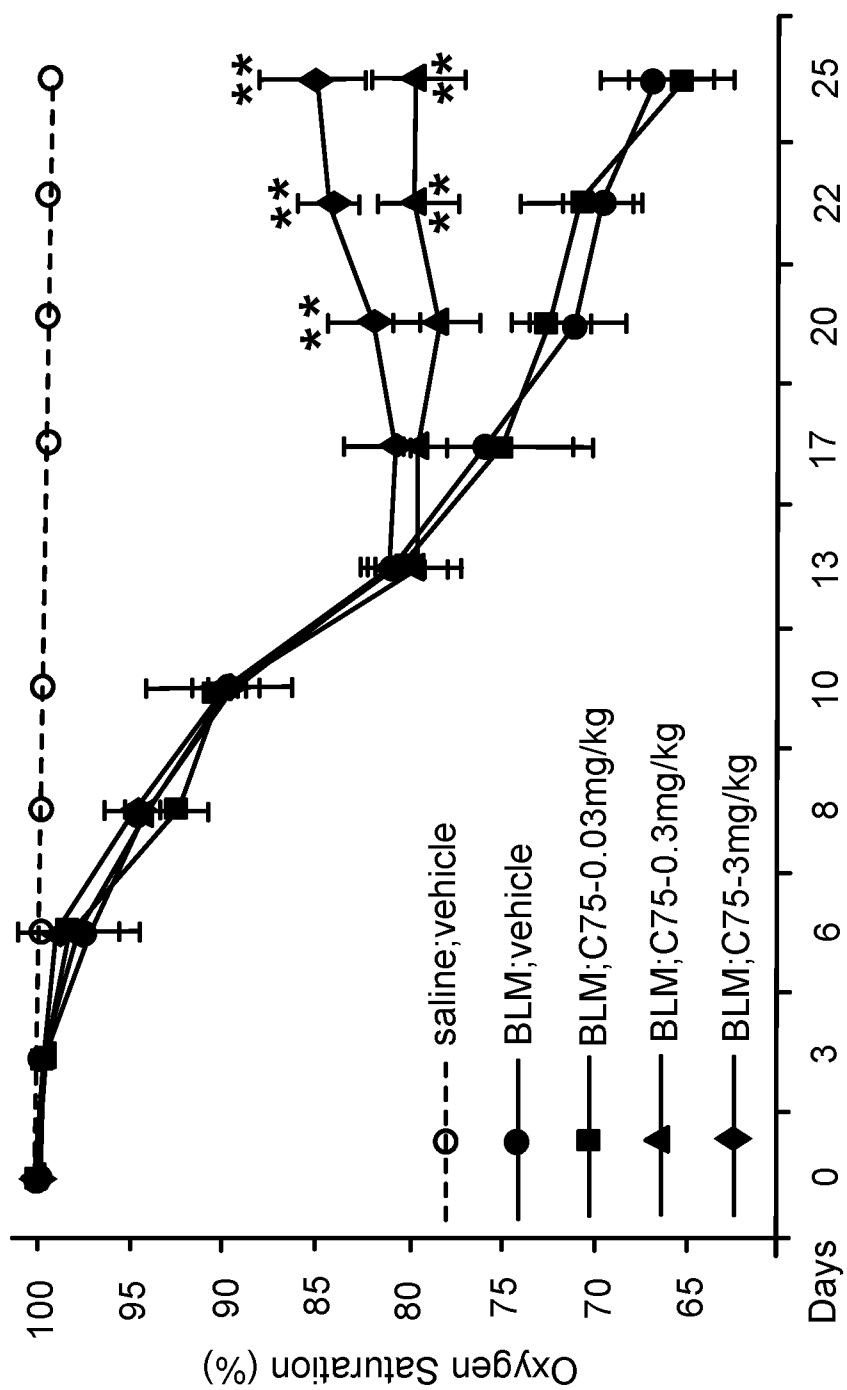


FIG. 9

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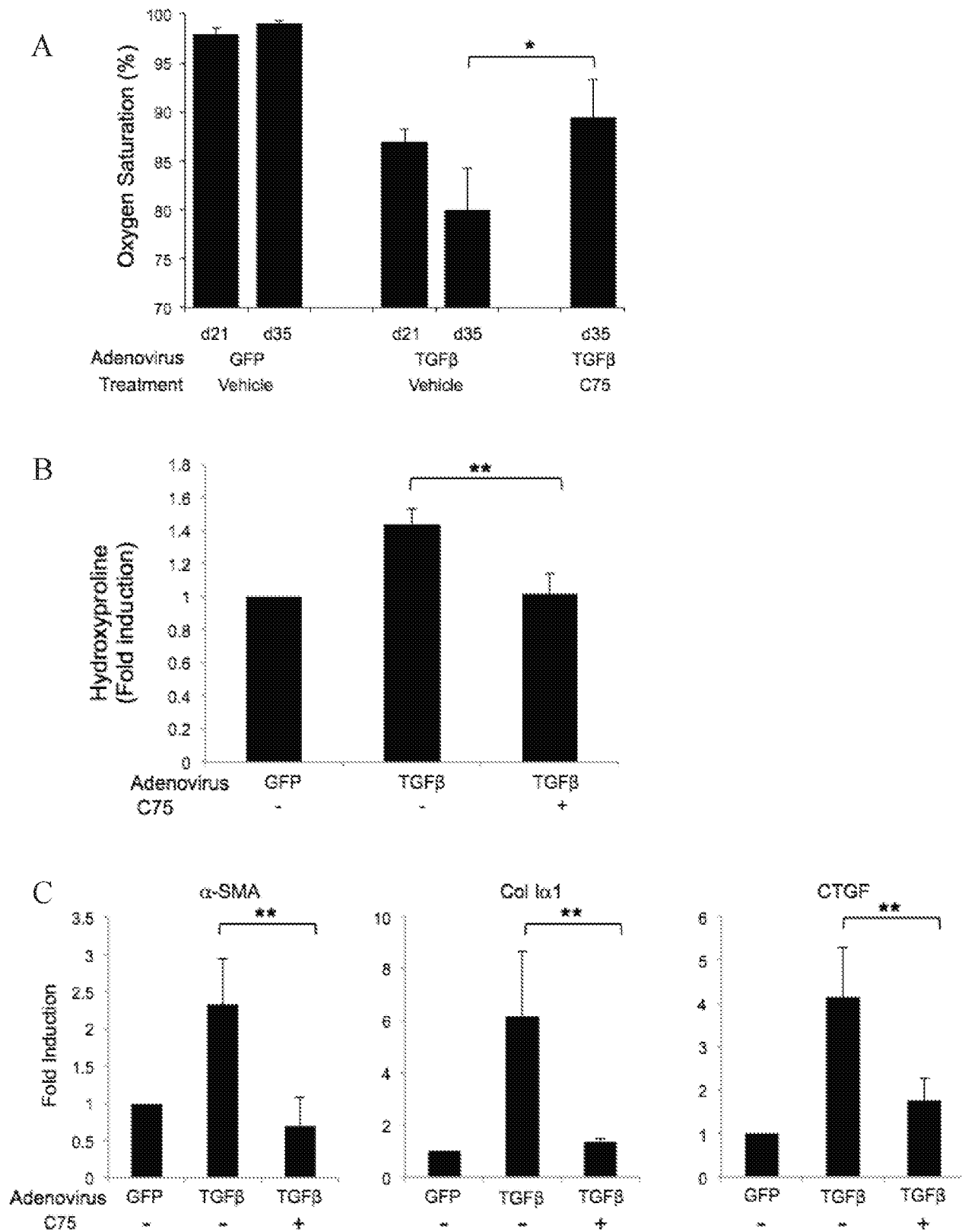


FIG. 10

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/23549

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/34; C12N 15/11; A61K 48/00; A61K 38/00 (2017.01)

CPC - A61K 31/365; A61K 31/34; C12N 15/113; A61K 48/00; C07K 14/4712

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)

See Search History Document

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

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category*       | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                        | Relevant to claim No.     |
|-----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|
| X<br>-----<br>Y | US 2015/0157634 A1 (PHARMACYCLICS, INC.) 11 June 2015 (11.06.2015), Abstract, para [0004], [0005], [0020], [0136], [0139], [0146], [0150], [0167], [0345], [0361], and [0363]                                                                                                                             | 1-2, 7-8<br>-----<br>3, 9 |
| Y               | US 2010/0190856 A1 (COLOMER BOSCH et al.) 29 July 2010 (29.07.2010), Abstract, para [0003], [0006], [0013], [0015], [0099], [0104], and [0222]                                                                                                                                                            | 3, 9                      |
| A               | CHEN et al., 4-Methylene-2-octyl-5-oxotetrahydrofuran-3-carboxylic Acid (C75), an Inhibitor of Fatty-acid Synthase, Suppresses the Mitochondrial Fatty Acid Synthesis Pathway and Impairs Mitochondrial Function. J Biol Chem. 2014, Vol. 289(24), p. 17184-94. Entire documentation, especially Abstract | 1-3, 7-9                  |
| A               | BAN et al., Fibrosis in diabetes complications: Pathogenic mechanisms and circulating and urinary markers. Vasc Health Risk Manag. 2008, Vol. 4(3), p. 575-5. Entire documentation, especially Abstract; pg 575, para 1; pg 577, col 1, lower para and Fig 1; pg 578, Fig 2; and pg 588, Fig 5            | 1-3, 7-9                  |
| A               | US 2010/0197708 A1 (TALLEY et al.) 05 August 2010 (05.08.2010), para [0134], [0135], [0152], [4103], [4016], [4054], and [4103]                                                                                                                                                                           | 1-3, 7-9                  |

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;" document member of the same patent family

Date of the actual completion of the international search

08 May 2017

Date of mailing of the international search report

17 JUL 2017

Name and mailing address of the ISA/US

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Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300  
PCT OSP: 571-272-7774

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/23549

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

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

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
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:
3. ☒ Claims Nos.: 5-6, 11-13, 17-19  
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:  
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-4, 7-10, directed to a method for treating fibrosis, or a method for treating a complication associated with fibrosis in a mammal, said method comprising: administering an inhibitor of fatty acid synthase activity. The inhibitor of fatty acid synthase will be searched to the extent that the inhibitor of fatty acid synthase encompasses wherein said inhibitor is C75. It is believed that claims 1-3, 7-9 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass wherein said inhibitor is C75. Additional inhibitor of fatty acid synthase will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected the inhibitor of fatty acid synthase. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be wherein said inhibitor is a fatty acid synthase targeting small interfering RNA [Claims (1-2), 4, (7-8), 10]. \*\*\*\*\*Continued in the extra 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 claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-3, 7-9, limited to C75

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/23549

Continuation of:  
Box No III (unity of invention is lacking)

Group II, claims 14-16, directed to a method for decreasing polypeptide expression in cells.

Group III, claims 20-23, directed to a method for improving peripheral blood oxygenation in a mammal.

The inventions listed as Groups I+ and II-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

## Special Technical Feature

Groups I+ include the special technical feature of treating fibrosis or treating complication associated with fibrosis, not required by Groups II-III.

Group II includes the special technical feature of decreasing polypeptide expression in cells, wherein expression of one or more profibrotic genes is decreased, not required by Groups I+ and III.

Group III includes the special technical feature of improving peripheral blood oxygenation, not required by Groups I+ and II.

Among Groups I+, an inhibitor of fatty acid synthase that is a chemical compound C75, is structurally and functionally different from an inhibitor of fatty acid synthase that is a fatty acid synthase targeting small interfering RNA, and vice versa. [Please see an article entitled '4-Methylene-2-octyl-5-oxotetrahydrofuran-3-carboxylic Acid (C75), an Inhibitor of Fatty-acid Synthase, Suppresses the Mitochondrial Fatty Acid Synthesis Pathway and Impairs Mitochondrial Function' by Chen et al.; J Biol Chem. 2014, Vol. 289(24), p. 17184-94 for the chemical name of C75: Abstract - '4-Methylene-2-octyl-5-oxotetrahydrofuran-3-carboxylic acid (C75) is a synthetic fatty-acid synthase (FASN) inhibitor']

## Common Technical Features

The inventions of Groups I+ and II-III share the technical feature of using (Groups I+ and III: administering; Group II, contacting) an inhibitor of fatty acid synthase activity;

--the inventions of Groups I+ and III further share the technical feature of a method for modulating a condition (Groups I+: treating fibrosis in a mammal, or treating a complication associated with fibrosis in a mammal; Group III: improving peripheral blood oxygenation in a mammal), the method comprising: administering an inhibitor of fatty acid synthase activity to a mammal; and

--the inventions of Groups I+ further share the technical feature of a method for treating fibrosis in a mammal, said method comprising: administering an inhibitor of fatty acid synthase activity to a mammal identified as having fibrosis; wherein a symptom of said fibrosis is reduced (claim 1), or

--a method for treating a complication associated with fibrosis in a mammal, said method comprising: administering an inhibitor of fatty acid synthase activity to a mammal identified as having a complication associated fibrosis; wherein a symptom of said complication is reduced (claim 7).

However, these shared technical features do not represent a contribution over prior art as being anticipated by US 2010/0190856 A1 to COLOMER BOSCH et al. (hereinafter 'COLOMER'); or as being obvious over US 2015/0157634 A1 to PHARMACYCLICS, INC. (hereinafter 'PHARMACYCLICS') as follows:

COLOMER discloses a method for modulating a condition (cancer or weight loss) (para [0104] - 'treatment of tumour cells expressing fatty acid synthase (FASN) and/or of a condition responsive to reduction in adipose tissue mass or for treatment to induce weight loss'; Abstract - 'fatty acid synthase (FASN)'), the method comprising:

--administering an inhibitor of fatty acid synthase activity to a mammal (Abstract - 'new polyhydroxylated compounds and, in particular, to its activity as fatty acid synthase (FASN) inhibitors ... for the treatment of pathological states for which an inhibitor of this enzyme is indicated'; para [0006] - 'FASN inhibitors ... for inducing weight loss and for inhibiting the growth of pre-existing cancer cells'; para [0015] - 'a method of treatment and/or prophylaxis of a mammal, including a human, suffering from or being susceptible to any of the diseases... administering to said patient a therapeutically effective amount of a compound of formula (I)'; para [0222] - 'FASN inhibitors (C75 and EGCG) induced apoptosis'; para [0003] - 'Compound C75 ((2R,3S)-4-methylene-2-octyl-5-oxotetrahydrofuran-3-carboxylic acid)').

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Continuation of:

The previous extra sheet - Box No III (unity of invention is lacking)

Furthermore, PHARMACYCLICS discloses a method for treating a disorder associated with fibrosis in a mammal (Abstract - 'treating ...chronic graft versus host disease (cGVHD)'; para [0363] - 'pulmonary fibrosis caused by cGVHD'; para [0020] - 'the cGVHD is treatment naive cGVHD...cGVHD is lung cGVHD. ... liver cGVHD. ... stomach cGVHD... fibrosis is reduced. ...lung fibrosis is reduced. ... liver fibrosis is reduced'; para [0136] - 'terms "individual", "patient" and "subject" ... refer to a mammal (e.g., a human) which is the object of treatment, or observation'), said method comprising:

--administering an inhibitor of fatty acid synthase activity to a mammal identified as having fibrosis (para [0005] - 'treating ... chronic graft versus host disease (cGVHD) in a patient ...administering a therapeutically effective amount of an ACK inhibitor'; para [0167] - 'ACK inhibitor is administered in combination with ...fatty acid synthase inhibitors'; para [0020] - 'the patient exhibits one or more symptoms of cGVHD ... fibrosis');

--wherein a symptom of said fibrosis is reduced (para [0020] - 'the patient exhibits one or more symptoms of cGVHD ...the cGVHD is treatment naive cGVHD...cGVHD is lung cGVHD. ... liver cGVHD. ... stomach cGVHD... fibrosis is reduced. ...lung fibrosis is reduced. ... liver fibrosis is reduced'; para [0150] - 'treating ... chronic graft versus host disease (cGVHD) in a patient...administering to the patient a composition comprising a therapeutically-effective amount of a BTK inhibitor,... fibrosis is reduced. ...lung fibrosis is reduced.... liver fibrosis is reduced'; para [0139] - 'treating ...chronic graft versus host disease (cGVHD) in a patient ... administering to the patient a composition comprising a therapeutically-effective amount of an ACK inhibitor compound ...e.g.,...BTK inhibitor ... ibrutinib'; para [0363] - 'ibrutinib therapy ameliorates pulmonary fibrosis caused by cGVHD (p<0.0001)'; para [0361] - 'Therapeutic Administration of Ibrutinib Ameliorated Pulmonary Fibrosis'; para [0167] - 'ACK inhibitor is administered in combination with ...fatty acid synthase inhibitors').

Although PHARMACYCLICS does not specifically teach using the method for treating fibrosis, one of the ordinary skill in the art at the time the invention was made would have known to use the method for treating a disorder associated with fibrosis for treating the fibrosis, to obtain a method for treating fibrosis in a mammal, said method comprising: administering an inhibitor of fatty acid synthase activity to a mammal identified as having fibrosis; wherein a symptom of said fibrosis is reduced, based on the teaching of PHARMACYCLICS, in order to apply the method taught by PHARMACYCLICS for facilitating treating associated different disorders including fibrosis with the disorders with a desired effect and without undue experimentation.

Furthermore, PHARMACYCLICS discloses a method for treating a complication that causes fibrosis in a mammal (Abstract - 'treating ... chronic graft versus host disease (cGVHD)'; para [0146] - 'GVHD is a major complication'; para [0004] - 'Chronic graft versus host disease (cGVHD) is the most common long-term complication following allogeneic stem cell transplant'; para [0363] - 'pulmonary fibrosis caused by cGVHD'; para [0020] - 'the cGVHD is treatment naive cGVHD...cGVHD is lung cGVHD. ... liver cGVHD. ... stomach cGVHD... fibrosis is reduced. ...lung fibrosis is reduced. ... liver fibrosis is reduced'; para [0136] - 'terms "individual", "patient" and "subject" ... refer to a mammal (e.g., a human) which is the object of treatment, or observation'), said method comprising:

--administering an inhibitor of fatty acid synthase activity to a mammal identified as having a complication associated fibrosis (para [0005] - 'treating ...chronic graft versus host disease (cGVHD) in a patient ...administering a therapeutically effective amount of an ACK inhibitor'; para [0167] - 'ACK inhibitor is administered in combination with ...fatty acid synthase inhibitors'; para [0004] - 'Chronic graft versus host disease (cGVHD) is the most common long-term complication'; para [0020] - 'the patient exhibits one or more symptoms of cGVHD ... fibrosis');

--wherein a symptom of said complication is reduced (para [0020] - 'the patient exhibits one or more symptoms of cGVHD ...the cGVHD is treatment naive cGVHD...cGVHD is lung cGVHD. ... liver cGVHD. ... stomach cGVHD... fibrosis is reduced. ...lung fibrosis is reduced. ... liver fibrosis is reduced...immunoglobulin (Ig) deposition in tissue is reduced'; para [0004] - 'Chronic graft versus host disease (cGVHD) is the most common long-term complication'; para [0150] - 'treating ... chronic graft versus host disease (cGVHD) in a patient ...administering to the patient a composition comprising a therapeutically-effective amount of a BTK inhibitor,... fibrosis is reduced.... immunoglobulin (Ig) deposition in tissue is reduced...the patient exhibits one or more symptoms of alloantibody driven cGVHD'; para [0345] - 'treatments for any disorder that benefit by inhibition of BTK, or in which BTK is a mediator or contributor to the symptoms or cause'; para [0157] - 'treating a patient for alleviation of a, with alleviation of consequently developed chronic graft versus host disease (cGVHD), comprising administering to the patient ...a therapeutically effective amount of an ACK inhibitor compound ...e.g., ...BTK inhibitor ... ibrutinib'; para [0363] - 'ibrutinib therapy ameliorates pulmonary fibrosis caused by cGVHD (p<0.0001)'; para [0167] - 'ACK inhibitor is administered in combination with ...fatty acid synthase inhibitors').

Although PHARMACYCLICS does not specifically teach using the method for treating a complication associated with fibrosis (a complication caused by fibrosis, based on the definition of the Specification) (Specification: pg 1, ln 24-25 - 'a complication associated with fibrosis (e.g., hypoxia caused by pulmonary fibrosis)'), one of the ordinary skill in the art at the time the invention was made would have known to use the method for treating complication that causes fibrosis for treating a complication associated with fibrosis (a complication caused by fibrosis, based on the definition of the Specification, as discussed above), to obtain a method for treating a complication associated with fibrosis in a mammal, said method comprising: administering an inhibitor of fatty acid synthase activity to a mammal identified as having a complication associated fibrosis; wherein a symptom of said complication is reduced, based on the teaching of PHARMACYCLICS, in order to apply the method taught by PHARMACYCLICS for facilitating treating and reducing different symptoms associated with fibrosis with a desired effect and without undue experimentation.

Without a shared special technical feature, the inventions lack unity with one another.

Groups I+ and II-III therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Continuation of item 4: Claims 5-6, 11-13, 17-19 are not drafted in accordance with the second and third sentences of Rule 6.4 (a). These claims are improper multiple dependent claims.

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**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US 17/23549

Continuation of:  
The previous extra sheet - Box No III (unity of invention is lacking)

Note:

I) There is no disclosure of the chemical name for C75 in the application.