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(71) Applicants: **INSERM (INSTITUT NATIONAL DE LA SANTÉ ET DE LA RECHERCHE MÉDICALE)** [FR/FR]; 101, rue de Tolbiac, 75013 Paris (FR). **UNIVERSITÉ D'AIX MARSEILLE** [FR/FR]; 58 Boulevard Charles Livon, 13284 Marseille Cedex 07 (FR). **INSTITUT JEAN PAOLI & IRENE CALMETTES** [FR/FR]; 232 Boulevard Sainte-Marguerite, 13009 Marseille (FR). **CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE (CNRS)** [FR/FR]; 3, Rue Michel Ange, 75016 Paris (FR).

(72) Inventors: **TOMASINI, Richard**; INSTITUT PAOLI-CALMETTES - INSERM U 1068 27 BOULEVARD LEI ROURE - CS30059, 13273 MARSEILLE CEDEX 09 (FR). **NIGRI, Jérémy**; AIX MARSEILLE UNIVERSITE - U1068 163 avenue de Luminy, 13288 MARSEILLE CEDEX 09 (FR). **BRESSY, Christian**; Universtity of North Carolina at Charlotte (UNCC) - Dept. of Biological Sciences Woodward Hall 486B 9201 University City, CHARLOTTE, CAROLINE DU NORD 28223 (US).

(74) Agent: **COLLIN, Matthieu**; Inserm Transfert 7 rue Watt, 75013 PARIS (FR).

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(54) Title: METHODS FOR THE DIAGNOSIS AND TREATMENT OF PANCREATIC DUCTAL ADENOCARCINOMA ASSOCIATED NEURAL REMODELING

(57) Abstract: The present invention relates to the diagnosis and treatment of pancreatic ductal adenocarcinoma (PDA) associated neural remodeling (PANR). The inventors investigated the effects of the stromal compartment on PDA and PANR and the specific connection between the stromal compartment and the nerve system in PDA. Using PDA samples from human and endogenous mouse models, the inventors demonstrated that LIF (leukemia inhibitory factor) is overexpressed in PDA tissues compared to healthy pancreas while its receptors, LIFR and gp130, are expressed in intra-tumoral nerves. The inventors demonstrated that LIF expression and secretion induced Schwann cell migration, decreased proliferation and modulated their differentiation status. LIF also induced neuronal plasticity. The injection of LIF blocking antibody in endogenous PDA mice model reduces intra-tumoral nerve density. Furthermore, using human and mouse serum libraries, the inventors showed that LIF titer improves CA19.9 diagnostic value and is positively correlated with intra-tumoral nerve density. Thus, the present invention relates to LIF inhibitor for use in the treatment of PANR and a method of identifying a subject having or at risk of having or developing PANR, comprising measuring the expression level of LIF.

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METHODS FOR THE DIAGNOSIS AND TREATMENT OF PANCREATIC DUCTAL ADENOCARCINOMA ASSOCIATED NEURAL REMODELING

FIELD OF THE INVENTION:

The present invention relates to the diagnosis of pancreatic ductal adenocarcinoma (PDA) associated neural remodeling (PANR). The present invention also relates to methods and pharmaceutical compositions for the treatment of PDA associated neural remodeling (PANR).

BACKGROUND OF THE INVENTION:

Pancreatic ductal adenocarcinoma (PDA) is considered as one of the most serious cancers, with a quick and asymptomatic evolution leading to a very low survival rate in patients (1,2) Even with recent improvements (3), current treatments, mainly based on surgery and chemotherapies, have a limited impact on the patient's fate, in part due to impaired drug perfusion provoked by the stromal reaction surrounding tumor cells (4,5). Indeed, PDA is characterized by the presence of a predominant stroma (intra-tumoral microenvironment) composed of cancer associated fibroblasts (CAFs), immune, endothelial and nerve cells. These have all been reported as drastic modifiers of tumor cells' abilities thereby impacting on pancreatic tumor evolution and prognosis (6). However, recent advances based on the effects of the stromal compartment on PDA are limited, and their clinical translation remains difficult (7).

In addition to evidence showing the major implication of the stroma in PDA evolution and in therapeutic resistance, several studies have highlighted profound alterations of the neural compartment and its concrete impact on patient's fate and quality of life (8,9). These alterations, called PDA associated neural remodeling (PANR), result in higher nerve densities in PDA due to peripheral nerve fibers infiltration and axonogenesis (10,11). Recently, we highlighted, in a previous study, that the intra-tumoral microenvironment could be a cause of those profound alterations (12). Thus, deciphering the specific connection between stromal compartment and nerve system in PDA could uncover potential therapeutic targets and clinical tools that would limit the nervous system-related impact on PDA evolution that alter patients' fate (13,14).

Indeed, a direct consequence of this neural remodeling in PDA is the appearance of perineural invasion (PNI) events, marked by the cancer cell's capacity to invade pancreatic nerves present within the tumor (15,16). In PDA, PNI is considered as an indicator of an

aggressive tumor associated with local recurrence and metastasis, acute neuropathic pain and leading to bad prognosis (8,17,18). Interestingly, recent reports have highlighted the role of the intra-tumoral microenvironment (19,20), and inflammatory processes (21) as pro-inflammatory cytokines like IL-6,(22) in PANR. Despite this, molecular mechanisms allowing neural remodeling and PNI events in PDA remain poorly understood. Thus, in-depth molecular studies are required to improve our knowledge in this field, which could provide new therapeutic opportunities to impair PDA progression and associated symptoms that limit patients' access to chemotherapy (23,24) and negatively influence their outcome.

Here we demonstrate, in human and mice, that LIF has a direct role on PDA Associated Neural Remodeling. We observed that, within PDA, stromal cells, mainly macrophages and fibroblasts, have the ability to secrete LIF acting then on pancreatic neural compartment.

Indeed, LIF can induce migration and differentiation of Schwann cells and neural plasticity of dorsal root ganglia (DRG) neurons through modulation of the JAK/STAT3 intracellular signaling. Using endogenous mice model of PDA treated with LIF-blocking antibody, we revealed that LIF is important for PANR. In addition, high levels of LIF were detected in sera from humans and mice with PDA but not that from healthy individuals or patients with benign pancreatic diseases. Altogether, our data suggest that LIF is a potent biomarker for the diagnosis of PDA, and that the therapeutic targeting of LIF-induced signaling in PDA could limit PANR and improve patient outcome and quality of life.

There is no disclosure in the art of the role of LIF in PDA associated neural remodeling (PANR), and the use of LIF inhibitors in the treatment of PDA associated neural remodeling (PANR).

SUMMARY OF THE INVENTION:

The present invention relates to the diagnosis of pancreatic ductal adenocarcinoma (PDA) associated neural remodeling (PANR). The present invention also relates to methods and pharmaceutical compositions for the treatment of PDA associated neural remodeling (PANR).

DETAILED DESCRIPTION OF THE INVENTION:

The inventors investigated the effects of the stromal compartment on pancreatic ductal adenocarcinoma (PDA) and PDA associated neural remodeling (PANR). The inventors also investigated the specific connection between the stromal compartment and the nerve system in PDA. PDA is characterized by a large stroma and important peripheral nervous system modifications. In the present invention, the inventors provide in vitro and in vivo evidences

that LIF (leukemia inhibitory factor), a pro-inflammatory cytokine belonging to the IL-6 family, is involved in PDA associated neural remodeling (PANR). Using PDA samples from human and endogenous mouse models, the inventors demonstrated that LIF is overexpressed in PDA tissues compared to healthy pancreas while its receptors, LIFR and gp130, are expressed in intra-tumoral nerves. Although both cancer and stromal cells expressed LIF, only stromal cells showed the ability to secrete LIF in the extra-cellular medium. This secreted LIF induced Schwann cell migration, decreased proliferation and modulated their differentiation status, through the activation of the JAK/STAT3/AKT intracellular signaling. LIF also induced neuronal plasticity in dorsal root ganglia neurons by increasing the number of neurites and the soma area. Finally, the injection of LIF blocking antibody in endogenous PDA mice model reduces intra-tumoral nerve density supporting the important role of LIF in PANR. Furthermore, using human and mouse serum libraries, the inventors showed that LIF titer improves CA19.9 diagnostic value and is positively correlated with intra-tumoral nerve density. Altogether, the present invention highlight the potential use of LIF serum titrating as a valuable diagnostic tool, as well as a therapeutic strategy aiming to limit LIF's impact on PANR.

Therapeutic method

Accordingly, the present invention relates to a LIF inhibitor for use in the treatment of PDA associated neural remodeling (PANR) in a subject in need thereof.

As used herein, the term "subject" denotes a mammal. Typically, a subject according to the invention refers to any subject (preferably human) afflicted with pancreatic ductal adenocarcinoma (PDA).

As used herein, the term "treatment" or "treat" refer to both prophylactic or preventive treatment as well as curative or disease modifying treatment, including treatment of subjects at risk of contracting the disease or suspected to have contracted the disease as well as subjects who are ill or have been diagnosed as suffering from a disease or medical condition, and includes suppression of clinical relapse. The treatment may be administered to a subject having a medical disorder or who ultimately may acquire the disorder, in order to prevent, cure, delay the onset of, reduce the severity of, or ameliorate one or more symptoms of a disorder or recurring disorder, or in order to prolong the survival of a subject beyond that expected in the absence of such treatment. By "therapeutic regimen" is meant the pattern of treatment of an illness, e.g., the pattern of dosing used during therapy. A therapeutic regimen may include an induction regimen and a maintenance regimen. The phrase "induction regimen" or "induction period" refers to a therapeutic regimen (or the portion of a therapeutic

regimen) that is used for the initial treatment of a disease. The general goal of an induction regimen is to provide a high level of drug to a subject during the initial period of a treatment regimen. An induction regimen may employ (in part or in whole) a "loading regimen", which may include administering a greater dose of the drug than a physician would employ during a maintenance regimen, administering a drug more frequently than a physician would administer the drug during a maintenance regimen, or both. The phrase "maintenance regimen" or "maintenance period" refers to a therapeutic regimen (or the portion of a therapeutic regimen) that is used for the maintenance of a subject during treatment of an illness, e.g., to keep the subject in remission for long periods of time (months or years). A maintenance regimen may employ continuous therapy (e.g., administering a drug at a regular intervals, e.g., weekly, monthly, yearly, etc.) or intermittent therapy (e.g., interrupted treatment, intermittent treatment, treatment at relapse, or treatment upon achievement of a particular predetermined criteria [e.g., disease manifestation, etc.]).

As used herein, the term “pancreatic ductal adenocarcinoma” or “PDA” has its general meaning in the art and refers to pancreatic ductal adenocarcinoma such as revised in the World Health Organisation Classification C25.

The term “PDA associated neural remodeling” or “PANR” has its general meaning in the art and refers to conditions resulting in higher nerve densities in PDA due to peripheral nerve fibers infiltration and axonogenesis (9, 10). The term “PDA associated neural remodeling” also refers to alterations caused by the PDA intratumoral microenvironment (11), this includes increased neural density, hypertrophy and pancreatic neuritis, as well as intra and extrapancreatic perineural invasion (PNI) by cancer cells (7, 9). The term “PDA associated neural remodeling” also refers to neural remodelling which is clinically correlated with neuropathic pain (7). Accordingly, the LIF inhibitor of the present invention is thus particularly suitable for reducing neuropathic pain in a subject suffering from pancreatic ductal adenocarcinoma.

As used herein, the term “LIF” has its general meaning in the art and refers to the cytokine leukemia inhibitory factor, a member of interleukin (IL)-6 family (Nicolas and Babon, 2015). The term “LIF” also refers to a glycoprotein synthesised as a 202 amino acid precursor that is post-translationally processed into a 20 kDa form by removal of 22 amino acids from its N-terminus (Nicolas and Babon, 2015).

The term “LIF inhibitor” has its general meaning in the art and refers to a compound that selectively blocks or inactivates the LIF. The term “LIF inhibitor” also refers to a compound that selectively blocks the binding of LIF to its receptors (LIFR and gp130). The

term "LIF inhibitor" also relates to LIF complex component inhibitor. The term "LIF inhibitor" also refers to a compound able to prevent the action of LIF and LIF complex component for example by inhibiting the LIF controls of downstream effectors such as inhibiting the activation of the JAK/STAT3/AKT intracellular signaling. As used herein, the term "selectively blocks or inactivates" refers to a compound that preferentially binds to and blocks or inactivates LIF with a greater affinity and potency, respectively, than its interaction with the other sub-types of the interleukine family. Compounds that block or inactivate LIF, but that may also block or inactivate other interleukine sub-types, as partial or full inhibitors, are contemplated. The term "LIF inhibitor" also refers to a compound that inhibits LIF complex components expression. Typically, a LIF inhibitor is a small organic molecule, a polypeptide, an aptamer, an antibody, an oligonucleotide or a ribozyme.

Tests and assays for determining whether a compound is a LIF inhibitor are well known by the skilled person in the art such as described in Vernallis et al., 1997; WO03064463; WO2011124566.

LIF inhibitors are well-known in the art as illustrated by Vernallis et al., 1997; WO03064463; WO2011124566.

In one embodiment of the invention, LIF inhibitors include but are not limited to the dominant-negative LIF mutant (hLIF-05) (Vernallis et al., 1997; WO03064463) and anti-LIF inhibitory antibodies such as described in WO2011/124566; WO 93/23556.

In another embodiment, the LIF inhibitor of the invention is a compound inhibiting the LIF complex component such as LIFR and gp130 antagonists.

The term "LIF complex component" has its general meaning in the art and refers to the ternary complex or trimer that consists of the LIF bound to gp130 as well as to LIFR (Nicolas and Babon, 2015).

Accordingly, the present invention also relates to a compound which is selected from the group consisting of LIFR antagonist, LIFR expression inhibitor, gp130 antagonist and gp103 expression inhibitor for use in the treatment of PDA associated neural remodeling (PANR) in a subject in need thereof.

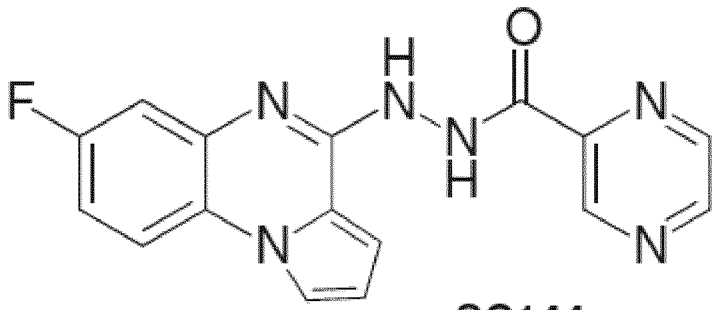
The term "LIFR" has its general meaning in the art and refers to the cytokine leukemia inhibitory factor (LIF) receptor. The term "LIFR" also refers to LIFRβ, a transmembrane signaling subunit that is structurally related to gp130 (Nicolas and Babon, 2015).

The term "LIFR antagonist" has its general meaning in the art and refers to compounds such as anti-LIFR antibodies and dominant-negative LIF mutant (hLIF-05) (Vernallis et al., 1997; WO03064463).

The term "gp130" has its general meaning in the art and refers to CD130, the cytokine leukemia inhibitory factor (LIF) subunit complex receptor (Nicolas and Babon, 2015).

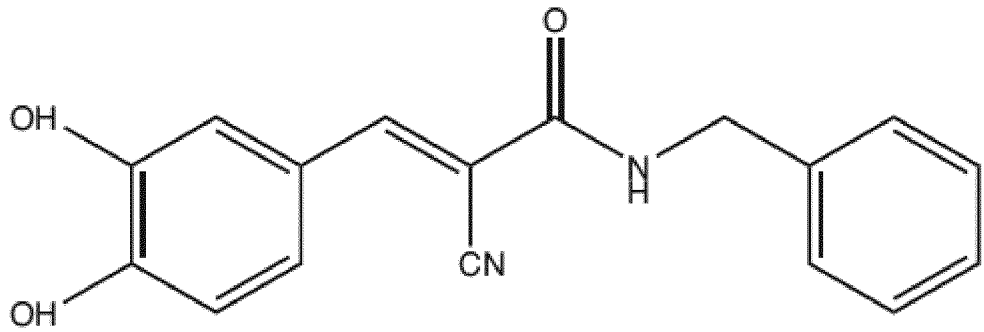
The term "gp130 antagonist" has its general meaning in the art and refers to compounds such as quinoxalinhydrazide derivative SC144 having the general formula (I), AG490 having the general formula (II), soluble forms of gp130 (sgp130) and compounds described in Seo et al., 2009; Xu et al., 2013; Huang et al., 2010; Fernandez-Botran, 2000; Xu and Neamati, 2013.

Formula I:



SC144

Formula II:



AG490

In some embodiments, the LIF inhibitor is a JAK/STAT3/AKT intracellular signaling inhibitor.

The term "JAK/STAT3/AKT intracellular signaling inhibitor" has its general meaning in the art and refers to compounds such as JAK inhibitors, STAT3 antagonists and AKT inhibitors.

JAK inhibitors such as JAK2 inhibitors are well known in the art (Tibes R, Bogenberger JM, Geyer HL, Mesa RA. JAK2 inhibitors in the treatment of myeloproliferative neoplasms. Expert Opin Investig Drugs. 2012 Dec;21(12):1755-74; Dymock BW, See CS. Inhibitors of JAK2 and JAK3: an update on the patent literature 2010 - 2012. Expert Opin

Ther Pat. 2013 Apr;23(4):449-501) and include but are not limited to ruxolitinib (INCB018424), SAR302503 (TG101348), Pacritinib (SB1518), CYT387, AZD-1480, BMS-911543, BMS-91153, NS-018, LY2784544, Lestaurtinib (CEP701), AT-9283, CP-690550, SB1578, R723, INCB16562, INCB20, CMP6, TG101209, SB1317 (TG02), XL-019, Baricitinib (LY3009104, INCB28050), AG490 and compounds described in WO2012030944, WO2012030924, WO2012030914, WO2012030912, WO2012030910, WO2010099379, WO2012030894, WO2010002472, WO2011130146, WO2010038060, WO2010020810, WO2011028864, WO2010141796, WO2010071885, WO2011101806, S20100152181, WO2010010190, WO2010010189, WO2010051549, WO2011003065, WO2012022265, WO2012068440, WO2011028685, WO2010135621, WO2010039939, WO2012068450, WO2011103423, WO2011044481, WO2010085597, WO2010014453, WO2010011375, WO2010069966, WO2011097087, WO2011075334, WO2011045702, WO2010020905, WO2010039518, WO2010068710.

The term "JAK inhibitor" also refers to JAK1 inhibitors such as ruxolitinib (INCB018424); GLPG-0634; Anilinophthalazine-based JAK1 inhibitors and compounds described in Norman, 2012; Nicolas and Babon, 2015; WO2010/135650; WO2011/086053; WO2009/152133; WO2011/068881; WO2011/112662; WO2012/037132.

STAT3 antagonists are well-known in the art as illustrated by Yu W., J Med Chem. 2013 May 7; Turkson et al., *Mol Cancer Ther.* 2004 Mar;3(3):261-9; McMurray JS. Chem Biol. 2006 Nov;13(11):1123-4; Liu A, Cancer Sci. 2011 Jul;102(7):1381-7; Song H., Proc Natl Acad Sci U S A. 2005 Mar 29;102(13); and Wang X., Int J Oncol. 2012 Jul;24.

The term "STAT3 antagonists" refers to compounds such as compounds that inhibit STAT3 phosphorylation such as PM-73G and pCinn-Leu-cis-3,4-methanoPro-Gln-NHBn (Yu W., J Med Chem. 2013 May 7); and non-peptidomimetic small inhibitors such as 5-hydroxy-9,10-dioxo-9,10-dihydroanthracene-1-sulfonamide (LLL12) and a steroidal natural product such as cucurbitacin (McMurray JS. Chem Biol. 2006 Nov;13(11):1123-4; Yu W., J Med Chem. 2013 May 7).

The term "STAT3 antagonists" also refers to compounds that inhibit STAT3 dimerization such as peptidomimetics XZH-5(Yu W., J Med Chem. 2013 May 7); ISS 610; ISS 219 and compounds described in Turkson et al., *Mol Cancer Ther.* 2004 Mar;3(3):261-9; and small molecules such as Stattic; STA-2; LLL-3; S3I-201 (NSC 74859); S3I-20; S3I-201.1066; S3I-M200; 5,15-DPP; STX-0119; Niclosamide (Siddiquee KA., ACS Chem Biol. 2007 Dec 21;2(12):787-98; Yu W., J Med Chem. 2013 May 7).

The term "STAT3 antagonists" refers to compounds such as 5,8-dioxo-6-(pyridin-3-ylamino)-5,8-dihydronaphthalene-1-sulfonamide (LY5); Naphthalene-5,8-dione-1-sulfonamide (Naphthalenesulfonylchloride); 5,8-dioxo-6-(phenylamino)-5,8-dihydronaphthalene-1-sulfonamide; 5*H*-Naphth[1,8-*cd*]isothiazol-5-one,1,1–dioxide,6-(phenylamino); 5*H*-Naphth[1,8-*cd*]isothiazol-5-one,1,1–dioxide,6-(1'-chloro-3'-nitro-2'-phenylamino); 5*H*-Naphth[1,8-*cd*]isothiazol-5-one,1,1–dioxide,6-(naphthylamino); Niclosamide (Yu W., J Med Chem. 2013 May 7); FLLL31; FLLL32 (Liu A, Cancer Sci. 2011 Jul;102(7):1381-7); NCT00511082; NCT00657176; NCT00955812; NCT01029509; NCT00696176 (Wang X., Int J Oncol. 2012 Jul 24).

In another embodiment, the LIF inhibitor of the invention is an aptamer. Aptamers are a class of molecule that represents an alternative to antibodies in term of molecular recognition. Aptamers are oligonucleotide sequences with the capacity to recognize virtually any class of target molecules with high affinity and specificity. Such ligands may be isolated through Systematic Evolution of Ligands by EXponential enrichment (SELEX) of a random sequence library, as described in Tuerk C. and Gold L., 1990. The random sequence library is obtainable by combinatorial chemical synthesis of DNA. In this library, each member is a linear oligomer, eventually chemically modified, of a unique sequence. Possible modifications, uses and advantages of this class of molecules have been reviewed in Jayasena S.D., 1999. Peptide aptamers consists of a conformationally constrained antibody variable region displayed by a platform protein, such as E. coli Thioredoxin A that are selected from combinatorial libraries by two hybrid methods (Colas et al., 1996). Then after raising aptamers directed against LIF of the invention as above described, the skilled man in the art can easily select those blocking or inactivating LIF.

In another embodiment, the LIF inhibitor of the invention is an antibody (the term including "antibody portion") directed against LIF, LIFR, gp130, or LIF complex component.

In one embodiment of the antibodies or portions thereof described herein, the antibody is a monoclonal antibody. In one embodiment of the antibodies or portions thereof described herein, the antibody is a polyclonal antibody. In one embodiment of the antibodies or portions thereof described herein, the antibody is a humanized antibody. In one embodiment of the antibodies or portions thereof described herein, the antibody is a chimeric antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a light chain of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a heavy chain of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the

antibody comprises a Fab portion of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a F(ab')2 portion of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a Fc portion of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a Fv portion of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a variable domain of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises one or more CDR domains of the antibody.

As used herein, "antibody" includes both naturally occurring and non-naturally occurring antibodies. Specifically, "antibody" includes polyclonal and monoclonal antibodies, and monovalent and divalent fragments thereof. Furthermore, "antibody" includes chimeric antibodies, wholly synthetic antibodies, single chain antibodies, and fragments thereof. The antibody may be a human or nonhuman antibody. A nonhuman antibody may be humanized by recombinant methods to reduce its immunogenicity in man.

Antibodies are prepared according to conventional methodology. Monoclonal antibodies may be generated using the method of Kohler and Milstein (Nature, 256:495, 1975). To prepare monoclonal antibodies useful in the invention, a mouse or other appropriate host animal is immunized at suitable intervals (e.g., twice-weekly, weekly, twice-monthly or monthly) with antigenic forms of LIF, LIFR, gp130, or LIF complex component. The animal may be administered a final "boost" of antigen within one week of sacrifice. It is often desirable to use an immunologic adjuvant during immunization. Suitable immunologic adjuvants include Freund's complete adjuvant, Freund's incomplete adjuvant, alum, Ribi adjuvant, Hunter's Titermax, saponin adjuvants such as QS21 or Quil A, or CpG-containing immunostimulatory oligonucleotides. Other suitable adjuvants are well-known in the field. The animals may be immunized by subcutaneous, intraperitoneal, intramuscular, intravenous, intranasal or other routes. A given animal may be immunized with multiple forms of the antigen by multiple routes.

Briefly, the antigen may be provided as synthetic peptides corresponding to antigenic regions of interest in LIF, LIFR, or gp130. Following the immunization regimen, lymphocytes are isolated from the spleen, lymph node or other organ of the animal and fused with a suitable myeloma cell line using an agent such as polyethylene glycol to form a hydridoma. Following fusion, cells are placed in media permissive for growth of hybridomas but not the fusion partners using standard methods, as described (Coding, Monoclonal Antibodies:

Principles and Practice: Production and Application of Monoclonal Antibodies in Cell Biology, Biochemistry and Immunology, 3rd edition, Academic Press, New York, 1996). Following culture of the hybridomas, cell supernatants are analyzed for the presence of antibodies of the desired specificity, i.e., that selectively bind the antigen. Suitable analytical techniques include ELISA, flow cytometry, immunoprecipitation, and western blotting. Other screening techniques are well-known in the field. Preferred techniques are those that confirm binding of antibodies to conformationally intact, natively folded antigen, such as non-denaturing ELISA, flow cytometry, and immunoprecipitation.

Significantly, as is well-known in the art, only a small portion of an antibody molecule, the paratope, is involved in the binding of the antibody to its epitope (see, in general, Clark, W. R. (1986) *The Experimental Foundations of Modern Immunology* Wiley & Sons, Inc., New York; Roitt, I. (1991) *Essential Immunology,* 7th Ed., Blackwell Scientific Publications, Oxford). The Fc' and Fc regions, for example, are effectors of the complement cascade but are not involved in antigen binding. An antibody from which the pFc' region has been enzymatically cleaved, or which has been produced without the pFc' region, designated an F(ab')2 fragment, retains both of the antigen binding sites of an intact antibody. Similarly, an antibody from which the Fc region has been enzymatically cleaved, or which has been produced without the Fc region, designated an Fab fragment, retains one of the antigen binding sites of an intact antibody molecule. Proceeding further, Fab fragments consist of a covalently bound antibody light chain and a portion of the antibody heavy chain denoted Fd. The Fd fragments are the major determinant of antibody specificity (a single Fd fragment may be associated with up to ten different light chains without altering antibody specificity) and Fd fragments retain epitope-binding ability in isolation.

Within the antigen-binding portion of an antibody, as is well-known in the art, there are complementarity determining regions (CDRs), which directly interact with the epitope of the antigen, and framework regions (FRs), which maintain the tertiary structure of the paratope (see, in general, Clark, 1986; Roitt, 1991). In both the heavy chain Fd fragment and the light chain of IgG immunoglobulins, there are four framework regions (FR1 through FR4) separated respectively by three complementarity determining regions (CDR1 through CDRS). The CDRs, and in particular the CDRS regions, and more particularly the heavy chain CDRS, are largely responsible for antibody specificity.

It is now well-established in the art that the non CDR regions of a mammalian antibody may be replaced with similar regions of conspecific or heterospecific antibodies while retaining the epitopic specificity of the original antibody. This is most clearly

manifested in the development and use of "humanized" antibodies in which non-human CDRs are covalently joined to human FR and/or Fc/pFc' regions to produce a functional antibody.

This invention provides in certain embodiments compositions and methods that include humanized forms of antibodies. As used herein, "humanized" describes antibodies wherein some, most or all of the amino acids outside the CDR regions are replaced with corresponding amino acids derived from human immunoglobulin molecules. Methods of humanization include, but are not limited to, those described in U.S. Pat. Nos. 4,816,567, 5,225,539, 5,585,089, 5,693,761, 5,693,762 and 5,859,205, which are hereby incorporated by reference. The above U.S. Pat. Nos. 5,585,089 and 5,693,761, and WO 90/07861 also propose four possible criteria which may used in designing the humanized antibodies. The first proposal was that for an acceptor, use a framework from a particular human immunoglobulin that is unusually homologous to the donor immunoglobulin to be humanized, or use a consensus framework from many human antibodies. The second proposal was that if an amino acid in the framework of the human immunoglobulin is unusual and the donor amino acid at that position is typical for human sequences, then the donor amino acid rather than the acceptor may be selected. The third proposal was that in the positions immediately adjacent to the 3 CDRs in the humanized immunoglobulin chain, the donor amino acid rather than the acceptor amino acid may be selected. The fourth proposal was to use the donor amino acid reside at the framework positions at which the amino acid is predicted to have a side chain atom within 3A of the CDRs in a three dimensional model of the antibody and is predicted to be capable of interacting with the CDRs. The above methods are merely illustrative of some of the methods that one skilled in the art could employ to make humanized antibodies. One of ordinary skill in the art will be familiar with other methods for antibody humanization.

In one embodiment of the humanized forms of the antibodies, some, most or all of the amino acids outside the CDR regions have been replaced with amino acids from human immunoglobulin molecules but where some, most or all amino acids within one or more CDR regions are unchanged. Small additions, deletions, insertions, substitutions or modifications of amino acids are permissible as long as they would not abrogate the ability of the antibody to bind a given antigen. Suitable human immunoglobulin molecules would include IgGl, IgG2, IgG3, IgG4, IgA and IgM molecules. A "humanized" antibody retains a similar antigenic specificity as the original antibody. However, using certain methods of humanization, the affinity and/or specificity of binding of the antibody may be increased using methods of "directed evolution", as described by Wu et al., /. *Mol. Biol.* 294:151, 1999, the contents of which are incorporated herein by reference.

Fully human monoclonal antibodies also can be prepared by immunizing mice transgenic for large portions of human immunoglobulin heavy and light chain loci. See, e.g., U.S. Pat. Nos. 5,591,669, 5,598,369, 5,545,806, 5,545,807, 6,150,584, and references cited therein, the contents of which are incorporated herein by reference. These animals have been genetically modified such that there is a functional deletion in the production of endogenous (e.g., murine) antibodies. The animals are further modified to contain all or a portion of the human germ-line immunoglobulin gene locus such that immunization of these animals will result in the production of fully human antibodies to the antigen of interest. Following immunization of these mice (e.g., XenoMouse (Abgenix), HuMAb mice (Medarex/GenPharm)), monoclonal antibodies can be prepared according to standard hybridoma technology. These monoclonal antibodies will have human immunoglobulin amino acid sequences and therefore will not provoke human anti-mouse antibody (KAMA) responses when administered to humans.

In vitro methods also exist for producing human antibodies. These include phage display technology (U.S. Pat. Nos. 5,565,332 and 5,573,905) and in vitro stimulation of human B cells (U.S. Pat. Nos. 5,229,275 and 5,567,610). The contents of these patents are incorporated herein by reference.

Thus, as will be apparent to one of ordinary skill in the art, the present invention also provides for F(ab') 2 Fab, Fv and Fd fragments; chimeric antibodies in which the Fc and/or FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric F(ab')2 fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric Fab fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; and chimeric Fd fragment antibodies in which the FR and/or CDR1 and/or CDR2 regions have been replaced by homologous human or non-human sequences. The present invention also includes so-called single chain antibodies.

The various antibody molecules and fragments may derive from any of the commonly known immunoglobulin classes, including but not limited to IgA, secretory IgA, IgE, IgG and IgM. IgG subclasses are also well known to those in the art and include but are not limited to human IgGl, IgG2, IgG3 and IgG4. In a preferred embodiment, the LIF inhibitor of the invention is a Human IgG4.

In another embodiment, the antibody according to the invention is a single domain antibody. The term “single domain antibody” (sdAb) or "VHH" refers to the single heavy chain variable domain of antibodies of the type that can be found in Camelid mammals which are naturally devoid of light chains. Such VHH are also called “nanobody®”. According to the invention, sdAb can particularly be llama sdAb. The term “VHH” refers to the single heavy chain having 3 complementarity determining regions (CDRs): CDR1, CDR2 and CDR3. The term “complementarity determining region” or “CDR” refers to the hypervariable amino acid sequences which define the binding affinity and specificity of the VHH.

The VHH according to the invention can readily be prepared by an ordinarily skilled artisan using routine experimentation. The VHH variants and modified form thereof may be produced under any known technique in the art such as in-vitro maturation.

VHHs or sdAbs are usually generated by PCR cloning of the V-domain repertoire from blood, lymph node, or spleen cDNA obtained from immunized animals into a phage display vector, such as pHEN2. Antigen-specific VHHs are commonly selected by panning phage libraries on immobilized antigen, e.g., antigen coated onto the plastic surface of a test tube, biotinylated antigens immobilized on streptavidin beads, or membrane proteins expressed on the surface of cells. However, such VHHs often show lower affinities for their antigen than VHHs derived from animals that have received several immunizations. The high affinity of VHHs from immune libraries is attributed to the natural selection of variant VHHs during clonal expansion of B-cells in the lymphoid organs of immunized animals. The affinity of VHHs from non-immune libraries can often be improved by mimicking this strategy in vitro, i.e., by site directed mutagenesis of the CDR regions and further rounds of panning on immobilized antigen under conditions of increased stringency (higher temperature, high or low salt concentration, high or low pH, and low antigen concentrations). VHHs derived from camelid are readily expressed in and purified from the E. coli periplasm at much higher levels than the corresponding domains of conventional antibodies. VHHs generally display high solubility and stability and can also be readily produced in yeast, plant, and mammalian cells. For example, the “Hamers patents” describe methods and techniques for generating VHH against any desired target (see for example US 5,800,988; US 5,874, 541 and US 6,015,695). The “Hamers patents” more particularly describe production of VHHs in bacterial hosts such as E. coli (see for example US 6,765,087) and in lower eukaryotic hosts such as moulds (for example Aspergillus or Trichoderma) or in yeast (for example Saccharomyces, Kluyveromyces, Hansenula or Pichia) (see for example US 6,838,254).

In one embodiment, the LIF inhibitor of the invention is a LIF complex components expression inhibitor such as LIF expression inhibitor, LIFR expression inhibitor and gp130 expression inhibitor.

The term "expression" when used in the context of expression of a gene or nucleic acid refers to the conversion of the information, contained in a gene, into a gene product. A gene product can be the direct transcriptional product of a gene (e.g., mRNA, tRNA, rRNA, antisense RNA, ribozyme, structural RNA or any other type of RNA) or a protein produced by translation of a mRNA. Gene products also include messenger RNAs, which are modified, by processes such as capping, polyadenylation, methylation, and editing, and proteins (e.g., LIF complex components such as LIF, LIFR and gp130) modified by, for example, methylation, acetylation, phosphorylation, ubiquitination, SUMOylation, ADP-ribosylation, myristilation, and glycosylation.

An "inhibitor of expression" refers to a natural or synthetic compound that has a biological effect to inhibit the expression of a gene.

LIF complex component expression inhibitors for use in the present invention may be based on antisense oligonucleotide constructs. Anti-sense oligonucleotides, including anti-sense RNA molecules and anti-sense DNA molecules, would act to directly block the translation of LIF complex components mRNA by binding thereto and thus preventing protein translation or increasing mRNA degradation, thus decreasing the level of LIF complex components proteins, and thus activity, in a cell. For example, antisense oligonucleotides of at least about 15 bases and complementary to unique regions of the mRNA transcript sequence encoding LIF complex components can be synthesized, e.g., by conventional phosphodiester techniques and administered by e.g., intravenous injection or infusion. Methods for using antisense techniques for specifically alleviating gene expression of genes whose sequence is known are well known in the art (e.g. see U.S. Pat. Nos. 6,566,135; 6,566,131; 6,365,354; 6,410,323; 6,107,091; 6,046,321; and 5,981,732).

Small inhibitory RNAs (siRNAs) can also function as LIF complex component expression inhibitors for use in the present invention. LIF complex component gene expression can be reduced by contacting the subject or cell with a small double stranded RNA (dsRNA), or a vector or construct causing the production of a small double stranded RNA, such that LIF complex component expression is specifically inhibited (i.e. RNA interference or RNAi). Methods for selecting an appropriate dsRNA or dsRNA-encoding vector are well known in the art for genes whose sequence is known (e.g. see Tuschl, T. et al. (1999); Elbashir, S. M. et al. (2001); Hannon, GJ. (2002); McManus, MT. et al. (2002);

Brummelkamp, TR. et al. (2002); U.S. Pat. Nos. 6,573,099 and 6,506,559; and International Patent Publication Nos. WO 01/36646, WO 99/32619, and WO 01/68836).

Ribozymes can also function as LIF complex component expression inhibitors for use in the present invention. Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Engineered hairpin or hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of LIF complex component mRNA sequences are thereby useful within the scope of the present invention. Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, which typically include the following sequences, GUA, GUU, and GUC. Once identified, short RNA sequences of between about 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site can be evaluated for predicted structural features, such as secondary structure, that can render the oligonucleotide sequence unsuitable. The suitability of candidate targets can also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using, e.g., ribonuclease protection assays.

Both antisense oligonucleotides and ribozymes useful a LIF complex component expression inhibitors can be prepared by known methods. These include techniques for chemical synthesis such as, e.g., by solid phase phosphoramadite chemical synthesis. Alternatively, anti-sense RNA molecules can be generated by in vitro or in vivo transcription of DNA sequences encoding the RNA molecule. Such DNA sequences can be incorporated into a wide variety of vectors that incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Various modifications to the oligonucleotides of the invention can be introduced as a means of increasing intracellular stability and half-life. Possible modifications include but are not limited to the addition of flanking sequences of ribonucleotides or deoxyribonucleotides to the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2'-O-methyl rather than phosphodiesterase linkages within the oligonucleotide backbone.

Antisense oligonucleotides siRNAs and ribozymes of the invention may be delivered in vivo alone or in association with a vector. In its broadest sense, a "vector" is any vehicle capable of facilitating the transfer of the antisense oligonucleotide siRNA or ribozyme nucleic acid to the cells and preferably cells expressing LIF complex component. Preferably, the vector transports the nucleic acid to cells with reduced degradation relative to the extent of

degradation that would result in the absence of the vector. In general, the vectors useful in the invention include, but are not limited to, plasmids, phagemids, viruses, other vehicles derived from viral or bacterial sources that have been manipulated by the insertion or incorporation of the antisense oligonucleotide siRNA or ribozyme nucleic acid sequences. Viral vectors are a preferred type of vector and include, but are not limited to nucleic acid sequences from the following viruses: retrovirus, such as moloney murine leukemia virus, harvey murine sarcoma virus, murine mammary tumor virus, and rouse sarcoma virus; adenovirus, adeno-associated virus; SV40-type viruses; polyoma viruses; Epstein-Barr viruses; papilloma viruses; herpes virus; vaccinia virus; polio virus; and RNA virus such as a retrovirus. One can readily employ other vectors not named but known to the art.

Preferred viral vectors are based on non-cytopathic eukaryotic viruses in which non-essential genes have been replaced with the gene of interest. Non-cytopathic viruses include retroviruses (e.g., lentivirus), the life cycle of which involves reverse transcription of genomic viral RNA into DNA with subsequent proviral integration into host cellular DNA. Retroviruses have been approved for human gene therapy trials. Most useful are those retroviruses that are replication-deficient (i.e., capable of directing synthesis of the desired proteins, but incapable of manufacturing an infectious particle). Such genetically altered retroviral expression vectors have general utility for the high-efficiency transduction of genes in vivo. Standard protocols for producing replication-deficient retroviruses (including the steps of incorporation of exogenous genetic material into a plasmid, transfection of a packaging cell lined with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral particles from tissue culture media, and infection of the target cells with viral particles) are provided in KRIEGLER (A Laboratory Manual," W.H. Freeman C.O., New York, 1990) and in MURRY ("Methods in Molecular Biology," vol.7, Humana Press, Inc., Cliffton, N.J., 1991).

Preferred viruses for certain applications are the adeno-viruses and adeno-associated viruses, which are double-stranded DNA viruses that have already been approved for human use in gene therapy. The adeno-associated virus can be engineered to be replication deficient and is capable of infecting a wide range of cell types and species. It further has advantages such as, heat and lipid solvent stability; high transduction frequencies in cells of diverse lineages, including hemopoietic cells; and lack of superinfection inhibition thus allowing multiple series of transductions. Reportedly, the adeno-associated virus can integrate into human cellular DNA in a site-specific manner, thereby minimizing the possibility of insertional mutagenesis and variability of inserted gene expression characteristic of retroviral

infection. In addition, wild-type adeno-associated virus infections have been followed in tissue culture for greater than 100 passages in the absence of selective pressure, implying that the adeno-associated virus genomic integration is a relatively stable event. The adeno-associated virus can also function in an extrachromosomal fashion.

Other vectors include plasmid vectors. Plasmid vectors have been extensively described in the art and are well known to those of skill in the art. See e.g., SANBROOK et al., "Molecular Cloning: A Laboratory Manual," Second Edition, Cold Spring Harbor Laboratory Press, 1989. In the last few years, plasmid vectors have been used as DNA vaccines for delivering antigen-encoding genes to cells in vivo. They are particularly advantageous for this because they do not have the same safety concerns as with many of the viral vectors. These plasmids, however, having a promoter compatible with the host cell, can express a peptide from a gene operatively encoded within the plasmid. Some commonly used plasmids include pBR322, pUC18, pUCl9, pRC/CMV, SV40, and pBlueScript. Other plasmids are well known to those of ordinary skill in the art. Additionally, plasmids may be custom designed using restriction enzymes and ligation reactions to remove and add specific fragments of DNA. Plasmids may be delivered by a variety of parenteral, mucosal and topical routes. For example, the DNA plasmid can be injected by intramuscular, intradermal, subcutaneous, or other routes. It may also be administered by intranasal sprays or drops, rectal suppository and orally. It may also be administered into the epidermis or a mucosal surface using a gene-gun. The plasmids may be given in an aqueous solution, dried onto gold particles or in association with another DNA delivery system including but not limited to liposomes, dendrimers, cochleate and microencapsulation.

Typically the inhibitors according to the invention as described above are administered to the subject in a therapeutically effective amount.

By a "therapeutically effective amount" of the inhibitor of the present invention as above described is meant a sufficient amount of the inhibitor for treating PDA associated neural remodeling (PANR) at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood, however, that the total daily usage of the inhibitors and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific inhibitor employed; the specific composition employed, the age, body weight, general health, sex and diet of the subject; the time of administration, route of administration, and rate of excretion of the specific inhibitor

employed; the duration of the treatment; drugs used in combination or coincidential with the specific inhibitor employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the inhibitor at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. However, the daily dosage of the products may be varied over a wide range from 0.01 to 1,000 mg per adult per day. Typically, the compositions contain 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100, 250 and 500 mg of the inhibitor of the present invention for the symptomatic adjustment of the dosage to the subject to be treated. A medicament typically contains from about 0.01 mg to about 500 mg of the inhibitor of the present invention, preferably from 1 mg to about 100 mg of the inhibitor of the present invention. An effective amount of the drug is ordinarily supplied at a dosage level from 0.0002 mg/kg to about 20 mg/kg of body weight per day, especially from about 0.001 mg/kg to 7 mg/kg of body weight per day.

In some embodiments, the LIF inhibitor of the present invention is administered to the subject in combination with anti-PDA treatment. The term "PDA treatment" has its general meaning in the art and refers to any type of pancreatic cancer therapy undergone by the pancreatic cancer subjects including surgical resection of pancreatic cancer, and any type of agent conventional for the treatment of PDA.

In some embodiments, the LIF inhibitor of the present invention is administered to the subject in combination with at least one compound selected from the group consisting of gemcitabine, fluorouracil, FOLFIRINOX (fluorouracil, irinotecan, oxaliplatin, and leucovorin), nab-paclitaxel, inhibitors of programmed death 1 (PD-1), PD-1 ligand PD-L1, anti-CLA4 antibodies, EGFR inhibitors such as erlotinib, chemoradiotherapy, inhibitors of PARP, inhibitors of Sonic Hedgehog, gene therapy and radiotherapy.

In a further aspect, the present invention relates to a method of screening a candidate compound for use as a drug for treating PDA associated neural remodeling (PANR) in a subject in need thereof, wherein the method comprises the steps of:

- providing a LIF, a LIFR, a gp130, providing a cell, tissue sample or organism expressing a LIF, a LIFR, a gp130,
- providing a candidate compound such as a small organic molecule, a polypeptide, an aptamer, an antibody or an intra-antibody,
- measuring the LIF activity,
- and selecting positively candidate compounds that inhibit LIF activity.

Methods for measuring LIF activity are well known in the art (Vernallis et al., 1997; WO03064463; WO2011124566). For example, measuring the LIF activity involves determining a Ki on the LIF cloned and transfected in a stable manner into a CHO cell line, measuring neuronal plasticity, measuring Schwann cell migration, measuring Schwann cell proliferation, measuring Schwann cell differentiation status and measuring JAK/STAT3/AKT intracellular signaling in the present or absence of the candidate compound.

Tests and assays for screening and determining whether a candidate compound is a LIF inhibitor are well known in the art (Vernallis et al., 1997; WO03064463; WO2011124566). In vitro and in vivo assays may be used to assess the potency and selectivity of the candidate compounds to inhibit LIF activity.

Activities of the candidate compounds, their ability to bind LIF, LIFR or gp130 and their ability to inhibit LIF activity may be tested using isolated Schwann cell or CHO cell line cloned and transfected in a stable manner by the human LIF, LIFR and gp130.

Activities of the candidate compounds and their ability to bind to the LIF, LIFR or gp130 may be assessed by the determination of a Ki on the LIF, LIFR and gp130 cloned and transfected in a stable manner into a CHO cell line, measuring neuronal plasticity, measuring Schwann cell migration, measuring Schwann cell proliferation, and measuring Schwann cell differentiation status in the present or absence of the candidate compound. The ability of the candidate compounds to inhibit LIF activity may be assessed by measuring LIF complex component formation, and measuring JAK/STAT3/AKT intracellular signaling such as described in the example.

Cells expressing another cytokine than LIF may be used to assess selectivity of the candidate compounds.

Pharmaceutical composition

The inhibitors of the invention may be used or prepared in a pharmaceutical composition.

In one embodiment, the invention relates to a pharmaceutical composition comprising the inhibitor of the invention and a pharmaceutical acceptable carrier for use in the treatment of PDA associated neural remodeling (PANR) in a subject of need thereof.

Typically, the inhibitor of the invention may be combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form therapeutic compositions.

"Pharmaceutically" or "pharmaceutically acceptable" refer to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when

administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

In the pharmaceutical compositions of the present invention for oral, sublingual, intramuscular, intravenous, local or rectal administration, the active principle, alone or in combination with another active principle, can be administered in a unit administration form, as a mixture with conventional pharmaceutical supports, to animals and human beings. Suitable unit administration forms comprise oral-route forms such as tablets, gel capsules, powders, granules and oral suspensions or solutions, sublingual and buccal administration forms, aerosols, implants, intraperitoneal, intramuscular, intravenous and intranasal administration forms and rectal administration forms.

Preferably, the pharmaceutical compositions contain vehicles which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

Solutions comprising inhibitors of the invention as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The inhibitor of the invention can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic

bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetables oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active inhibitors in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

In addition to the inhibitors of the invention formulated for parenteral administration, such as intravenous or intramuscular injection, other pharmaceutically acceptable forms include, e.g. tablets or other solids for oral administration; liposomal formulations; time release capsules; and any other form currently used.

Pharmaceutical compositions of the invention may include any further compound which is used in the treatment of pancreatic ductal adenocarcinoma.

In one embodiment, said additional active compounds may be contained in the same composition or administrated separately.

In another embodiment, the pharmaceutical composition of the invention relates to combined preparation for simultaneous, separate or sequential use in the treatment of PDA associated neural remodeling (PANR) in a subject in need thereof.

The invention also provides kits comprising the inhibitor of the invention. Kits containing the inhibitor of the invention find use in therapeutic methods.

Diagnostic method

A further aspect of the invention relates to a method of identifying a subject having or at risk of having or developing PDA associated neural remodeling (PANR), comprising a step of measuring in a biological sample obtained from said subject the expression level of LIF.

The term “biological sample” refers to any biological sample derived from the subject such as blood sample, plasma sample, serum sample or PDA sample.

The method of the invention may further comprise a step consisting of comparing the expression level of LIF in the biological sample with a reference value, wherein detecting differential in the expression level of LIF between the biological sample and the reference value is indicative of subject having or at risk of having or developing PDA associated neural remodeling (PANR).

As used herein, the “reference value” refers to a threshold value or a cut-off value. Typically, a "threshold value" or "cut-off value" can be determined experimentally, empirically, or theoretically. A threshold value can also be arbitrarily selected based upon the existing experimental and/or clinical conditions, as would be recognized by a person of ordinary skilled in the art. The threshold value has to be determined in order to obtain the optimal sensitivity and specificity according to the function of the test and the benefit/risk balance (clinical consequences of false positive and false negative). Typically, the optimal sensitivity and specificity (and so the threshold value) can be determined using a Receiver Operating Characteristic (ROC) curve based on experimental data. Preferably, the person skilled in the art may compare the expression level (obtained according to the method of the

invention) with a defined threshold value. In one embodiment of the present invention, the threshold value is derived from the expression level (or ratio, or score) determined in a biological sample derived from one or more subjects having PDA associated neural remodeling (PANR). Furthermore, retrospective measurement of the expression level (or ratio, or scores) in properly banked historical subject samples may be used in establishing these threshold values.

In one embodiment, the reference value may correspond to the expression level of LIF determined in a biological sample associated with a subject not afflicted with PDA associated neural remodeling (PANR). Accordingly, a higher expression level of LIF than the reference value is indicative of a subject having or at risk of having or developing PDA associated neural remodeling (PANR), and a lower or equal expression level of LIF than the reference value is indicative of a subject not having or not at risk of having or developing PDA associated neural remodeling (PANR).

In another embodiment, the reference value may correspond to the expression level of LIF determined in a biological sample associated with a subject afflicted with PDA associated neural remodeling (PANR). Accordingly, a higher or equal expression level of LIF than the reference value is indicative of a subject having or at risk of having or developing PDA associated neural remodeling (PANR), and a lower expression level of LIF than the reference value is indicative of a subject not having or not at risk of having or developing PDA associated neural remodeling (PANR).

Analyzing the LIF expression level may be assessed by any of a wide variety of well-known methods for detecting expression of a transcribed nucleic acid or translated protein.

In one embodiment, the LIF expression level is assessed by analyzing the expression of mRNA transcript or mRNA precursors, such as nascent RNA, of LIF gene. Said analysis can be assessed by preparing mRNA/cDNA from cells in a biological sample from a subject, and hybridizing the mRNA/cDNA with a reference polynucleotide. The prepared mRNA/cDNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or Northern analyses, polymerase chain reaction analyses, such as quantitative PCR (TaqMan), and probes arrays such as GeneChip(TM) DNA Arrays (AFFYMETRIX).

Advantageously, the analysis of the expression level of mRNA transcribed from the gene encoding for LIF involves the process of nucleic acid amplification, e. g., by RT-PCR (the experimental embodiment set forth in U. S. Patent No. 4,683, 202), ligase chain reaction (Barany, 1991), self sustained sequence replication (Guatelli et al., 1990), transcriptional

amplification system (Kwoh et al., 1989), Q-Beta Replicase (Lizardi et al., 1988), rolling circle replication (U. S. Patent No. 5,854, 033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers. As used herein, amplification primers are defined as being a pair of nucleic acid molecules that can anneal to 5' or 3' regions of a gene (plus and minus strands, respectively, or vice-versa) and contain a short region in between. In general, amplification primers are from about 10 to 30 nucleotides in length and flank a region from about 50 to 200 nucleotides in length. Under appropriate conditions and with appropriate reagents, such primers permit the amplification of a nucleic acid molecule comprising the nucleotide sequence flanked by the primers.

In another embodiment, the LIF expression level is assessed by analyzing the expression of the protein translated from said gene. Said analysis can be assessed using an antibody (e.g., a radio-labeled, chromophore- labeled, fluorophore-labeled, or enzyme-labeled antibody), an antibody derivative (e.g., an antibody conjugate with a substrate or with the protein or ligand of a protein of a protein/ligand pair (e.g., biotin-streptavidin)), or an antibody fragment (e.g., a single-chain antibody, an isolated antibody hypervariable domain, etc.) which binds specifically to the protein translated from the gene encoding for LIF.

Said analysis can be assessed by a variety of techniques well known from one of skill in the art including, but not limited to, enzyme immunoassay (EIA), radioimmunoassay (RIA), Western blot analysis and enzyme linked immunoabsorbant assay (RIA).

In a further aspect, the method of the invention is performed by measuring LIF activation level.

Analyzing the LIF activation level may be assessed by any of a wide variety of well-known methods (Vernallis et al., 1997; WO03064463; WO2011124566).

In one embodiment, the LIF activation level is assessed by measuring LIF, LIFR and pg130 heterodimerization or measuring JAK/STAT3/AKT intracellular signalling.

A further aspect of the invention relates to a method of monitoring PDA progression by performing the method of the invention.

In one embodiment, the present invention relates to a method of treating PDA associated neural remodeling (PANR) in a subject in need thereof comprising the steps of:

(i) identifying a subject having or at risk of having or developing a PDA associated neural remodeling (PANR) by performing the method according to the invention, and

(ii) administering to said subject a LIF inhibitor when it is concluded that the subject has or is at risk of having PDA associated neural remodeling (PANR).

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

FIGURES:

Figure 1. LIF, LIFR and gp130 expression levels in human and murine PDA. A, LIF immunoblot in human healthy pancreas (H, n=5) or PDA (P, n=2). Quantifications noted are expressed as fold increase compared with H1. **B**, Representative images following colour deconvolution of LIF staining in human healthy pancreas (n=6) or PDA (n=6) (bars, 50µm), with corresponding quantifications (mean ± SD). **C**, Fold change of *LIF* mRNA expression level in mouse healthy pancreas (n=6) or PDA (n=6); each dot is representative from one mouse. **D**, Representative images following colour deconvolution of LIF staining in mouse healthy pancreas (n=6) or PDA (n=6) (bars, 100µm), with corresponding quantifications (mean ± SD). **E**, LIF immunoblot in mouse healthy pancreas (H, n=5) or PDA (P, n=6). Quantifications noted are expressed as fold changes compared with H1. Each experiment was reproduced at least three times. *, $P < 0.05$; **, $P < 0.01$.

Figure 2. In PDA, LIF secretion is driven by stromal compartment and mainly by CAFs. A, Human (top panel) and mouse (bottom panel) *LIF* mRNA expression levels (mean ± SD). B, Human LIF mRNA expression levels (mean ± SD). PDA#1 to 4 represents human PDA primary tumor cells. **C**, LIF immunoblots. Quantifications noted are expressed as fold changes compared to macrophages (Raw) or fibroblasts (CAF). **D**, LIF immunoblots. Quantifications noted are expressed as fold changes compared to fibroblasts co-cultivated with macrophages (FHN+RAW). **E**, Quantification of secreted LIF, by ELISA assay, in various stromal cells conditioned media (mean ± SD). **F**, Quantification of secreted LIF, by ELISA assay, in conditioned media from stromal and tumor cells (mean ± SD). Each experiment was reproduced at least three times. *, $P < 0.05$; **, $P < 0.01$.

Figure 3. LIF-triggered signaling enhance migratory capacities of Schwann nerve cells. A to C, Effects of stromal cells conditioned media on sNF96.2 migration ability (A) (mean ± SD) using Ab-LIFR/Ab-Ctrl (B) or AG490/SC144 (30µM/2µM respectively, pre-incubation for 2h) (C). D Effects of various doses (0-320ng/ml) of LIF recombinant protein on sNF96.2 migration (mean ± SD). E, Impact of Ab-LIFR on sNF96.2 migration (mean ± SD), using 50ng/ml of LIF and various doses of Ab-LIFR (upper panel) or 4µg/ml of Ab-LIFR compared to Ab-Ctrl (lower panel). F, Impact of AG490/SC144 on sNF96.2 migration

(mean ± SD), using 50ng/ml of LIF. G, pSTAT3 and pAKT immunoblots in sNF96.2 cells following CM incubations and AG490/SC144 treatments. Quantifications noted are expressed as fold changes compared with sNF96.2 cells under sNF96.2 media. Each experiment was reproduced at least three times. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Figure 4. LIF reduces Schwann cells proliferation. A to C, Cell count of sNF96.2 cells incubated with stromal conditioned media (A) together with Ab-LIFR (B) or LIF recombinant protein (C) (mean ± SD). D, Effect of LIF recombinant protein (50ng/ml) on *p21* mRNA expression in sNF96.2 cells (mean ± SD). E, p21 immunoblots from sNF96.2 incubated for 36 (top panel) or 48 (down panel) hours with 50ng/ml of LIF. Quantifications noted are expressed as fold changes compared with sNF96.2 cells not incubated with LIF recombinant protein. F and G, p21 immunoblots from sNF96.2 incubated for 36 hours with 50ng/ml of LIF (F) or various conditioned media (G) together with AG490 (F and G) or SC144 (F) treatments. Each experiment was reproduced at least three times. *, $P < 0.05$.

Figure 5. LIF induces Schwann cell differentiation and neural plasticity. Pou3F2 and S100 dual-staining in sNF96.2 cells incubated with control (sNF96.2) or stromal conditioned media, in presence of Ab-Ctrl or Ab-LIFR. **A**, S100 and Pou3F2 immunoblots in sNF96.2 cultured cells. **B**, S100 and Pou3F2 mRNA expression levels in sNF96.2 cells incubated with 50ng/ml of LIF recombinant protein (mean ± SD). **C**, S100 and Pou3F2 immunoblots from sNF96.2 cells incubated for 48 hours with LIF (50ng/ml). **D** and **E**, Effects of 50ng/ml of LIF (24 and 48 hrs) on neuronal plasticity (**D**, neurite number and **E**, soma area) of neurons from DRG (mean ± SD). Each experiment was reproduced at least three times. *, $P < 0.05$.F

Figure 6. LIF is a potent diagnostic and predictive biomarker for PDA. A, Measurement of LIF level in serum from healthy (n=12), acute (n=10) or chronic (n=9) pancreatitis as well as PDA bearing (n=12) mice. **B**, Linear regression of intra-PDA nerve number versus LIF titer in PDA bearing mice sera (n=12). **C**, Measurement of serum LIF level and intra-tumoral nerve number in PDA-bearing mice treated with control-antibody (n=6, [LIF]<124pg/ml and n=6, [LIF]>124pg/ml) or LIF neutralizing antibody (n=9, [LIF]>124pg/ml). **D**, Measurement of LIF level in human serum from healthy donors (n=61), chronic pancreatitis (n=31), benign pancreatic tumor (n=11) and PDA (n=142) patients. **E**, Linear regression of intra-tumoral nerve number versus LIF titer in serum from PDA patients (n=10). **F**, Receiver operating characteristic (ROC) curve analyses from serum level of CA 19-9, with AUC = 0.901, and CA19-9+LIF, with AUC = 0.984. Analysis realized on a control group involving healthy donors (n=57) with chronic pancreatitis patients (n=27) versus 64

PDA patients (n=64). **G**, Graphical representation summarizing the impact of stromal secreted LIF on PANR and its potent use as a biomarker.

Figure 7. LIF expression level in fibroblasts (FHN) and primary CAFs from human PDA. *LIF* mRNA expression level in FHN and CAFs extracted from freshly resected PDA (n=9) (mean ± SD). Quantifications noted are expressed as fold increase compared with FHN. The experiment was reproduced at least three times. *, $P < 0.05$.

Figure 8. Graphical representations of co-culture method and migration assay. A, Method of cell co-culture in order to produce specific conditioned media (CM). Those CM are used in the following migration assay or for LIF ELISA tittering while cells from the co-culture are then recovered for protein or mRNA extracts. **B**, Migration assay method for the measurement of migration ability of sNF96.2 cells using various conditioned media (CM).

Figure 9. LIF induces activation of AKT/STAT3 signaling pathways in Schwann cells. A, pSTAT3 immunoblot in sNF96.2 cells following incubations with human LIF recombinant protein. Quantifications noted are expressed as fold changes compared with sNF96.2 cells untreated. **B**, pAKT immunoblot in sNF96.2 cells following incubations with human LIF recombinant protein. Quantifications noted are expressed as fold changes compared with sNF96.2 cells untreated. **C**, pSTAT3 and pAKT immunoblots in sNF96.2 cells following incubations with human LIF recombinant protein and treatments with AG490 or Ab-LIFR. Quantifications noted are expressed as fold changes compared with sNF96.2 cells untreated. Each experiment was reproduced at least three times.

Figure 10. LIF do not modify Schwann cell survival. A, Quantification of caspase positive sNF96.2 cells following 48 hours incubation with human LIF recombinant protein (mean ± IQR). Quantifications noted are expressed as fold increase compared with untreated cells. B, Quantification of caspase positive sNF96.2 cells following 48 hours incubation with conditioned media from FHN+RAW or FHN+RAW+HMC-1 (mean ± IQR). Quantifications noted are expressed as fold increase compared with cells incubated with sNF96.2 media. Each experiment was reproduced at least three times. ns, not significant.

EXAMPLE:

Material & Methods

Cell lines

Human pancreatic cancer cell lines (PANC-1, MIApaCa-2, BxPC-3, Capan-2) and Schwann cells (sNF96.2) as well as murine macrophage (RAW264.7) were obtained from American Type Culture Collection (ATCC) and cultivated in DMEM medium supplemented with 10% fetal bovine serum (Life Technologies) and 1% of antibiotic/antimycotic

(Invitrogen, 15240-062). Human mast cells (HMC-1) were provided by Professor Michel Arock (ENS Cachan, France) and cultivated in RPMI-1640 medium, supplemented with 10% fetal bovine serum and 1% of antibiotic/antimycotic. Human primary fibroblasts (FHN) were a kind gift from Dr Cedric Gaggioli (IRCAN, Nice, France) and CAF cells (produced from freshly resected human PDA) (25) were cultivated in DMEM medium and DMEM/F12 medium respectively. PDA-1 to 4 are human pancreatic primary cancer cells derived from freshly resected PDA samples (26). All patients gave their consent and are included in the clinical trial number 2011-A01439-32 (26). Expert clinical centers collaborated on this project after approval from their respective ethics review board (approval number 11-61).

Human samples

Chronic pancreatitis (31 samples), pancreatic benign tumor (11 samples) or PDAC (142 samples) sera or tissues used for elisa assay, immunostaining or immunoblots were collected in patients from Hôpital Nord and La Timone, Marseille, France but also from Hôpital La Pitié-Salpêtrière, Paris, France. In addition, 61 healthy donors samples were amassed from Hôpital La Timone, Marseille, and from Etablissement Français du Sang (EFS), Marseille, France. All Patients were recruited to participate in a translational research study of blood samples. They accepted and signed an informed consent that had been approved by the local ethics committee (Agreement reference of CRO2 for tissue collection: DC-2013-1857). Concerning PDA patients that underwent surgical resections, PDA specimens were routinely fixed in 10% formalin, embedded in paraffin and further cut into 5 mm sections immediately stored at 4°C or stained with hematoxylin-phloxine-saffron (HPS). All tissues were collected via standardized operative procedures approved by the Institutional Ethical Board and in accordance with the Declaration of Helsinki. Informed consent was obtained for all tissue samples linked with clinical data.

Murine serum

Sera from healthy and PDA bearing mice were obtained after intra-cardiac puncture and separation between plasma and blood cells by centrifugation. Mice developing PDA were euthanized when they were moribund (average of 8.5 weeks old).

Statistical Analysis

The results showed are averages or medians, and error bars in graphs represent standard deviations (SD). The Mann–Whitney test, recommended for the comparison of two independent groups, was performed when required. The Wilcoxon test was used, when required, to analyze two different parameters within an experimental group. Differences were considered significant if P was less than 0.05. All P values were calculated using the

Graphpad prism software. All experiments were repeated at least 3 times. ROC curves and AUC were computed using the ROCR package and statistical analyses were computed using the statistical software environment R, version 3.2.1. CA19-9, LIF and IL-6 concentrations were transformed using a lin-log function (namely arc-sinus-hyperbolic) and scaled in order to cover the same range. Combining markers was carried out by the estimation of a logistic model. The rms package allowed logistic regression and models comparison.

Mouse model

Pdx1-cre;Ink4a/Arf$^{fl/fl}$;LSL-Kras^{G12D} (50) mice were obtained by crossing the following strains: Pdx1-cre, Kras^{G12D} and Ink4A$^{fl/fl}$ mice kindly provided by Dr. D Melton (Harvard Stem Cell Institute, Cambridge, MA, USA), Dr. R Depinho (Dana-Farber Cancer Institute, Boston, MA, USA) and Dr T Jacks (David H Koch Institute for Integrative Cancer Research, Cambridge, MA, USA), respectively. Pieces of tumor pancreas were fixed in 4% (wt/vol) formaldehyde for further immunostaining analysis or prepared for RNA extraction. All animal care and experimental procedures were performed in agreement with the Animal Ethics Committee of Marseille.

Establishment of acute and chronic pancreatitis in mouse

Acute pancreatitis in mice was realized by injection of Caerulein (50µg/kg/100µl, from AnaSpec As-24252) by intraperitoneal mode 6 times per hour during 6 hours. Sera and pancreas was removed 2 hours after the last injection. Chronic pancreatitis was induced by twice a week injection of Caerulein (50µg/kg/100µl) by intraperitoneal mode for 10 weeks.

Reagents

Human recombinant leukemia inhibitory factor (LIF) was obtained from Prospec (CYT-644, 25µg). Several LIF pathway inhibitors were used: Ab-LIFR, a LIF receptor blocking antibody (Santa-cruz biotechnology: sc-659, C19 clone) used at 4µg/ml; tyrphostin (AG490), a JAK-2 protein tyrosine kinase (Sigma-Aldrich, T3434-5MG) used at 30µM; and SC144 hydrochloride (Sigma-Aldrich, SML0763-5MG), an inhibitor of glycoprotein gp130 (gp130) used at 2µM. A control polyclonal antibody from rabbit anti-rat IgG (Ba1042.5 Boster biological technology) was used for in vitro experience at 4µg/ml. For in vivo experiments, an anti-LIF neutralizing antibody produced in goat, IgG fraction of antiserum was used (L9152, Sigma-Aldrich). Antibody was injected (20µg/kg/souris/IP/100µl) by intraperitoneal mode twice a week during 4 weeks in PDAC mice aged of 4 weeks old.

Conditioned media (CM) were collected from cell cultures. 300. 000 sNF96.2, RAW, HMC-1, FHN were cultivated together alone or in association (300 000 for each cellular type) in Multiwell 6 plates. After 24h of culture in cellular medium containing 0.5% FBS,

conditioned media were centrifuged at 1200 rpm and cell supernatants were harvested and used in different *in vitro* assays.

Cell migration assay

Migration assays were performed with sNF96.2 cells as described in a previous study (12).

Cell proliferation

sNF96.2 cells ($1.5x10^5$) were plated on 6-well plates. The next day, normal medium was changed with either various conditioned media from stromal or nerve cells (FHN, RAW, HMC-1 or sNF96.2) in a DMEM medium containing 0.5% FBS supplemented, or not, with LIF (50ng/ml). Cell proliferation was measured at different time points (24h or 48h later) by trypan blue counting using a ViCELL XR (Beckman Coulter).

Elisa Assay

LIF titration, in cell supernatant or in serum, was performed with the LIF ELISA Kit according to the supplier's instructions (Raybiotech, ELH-LIF-001). Sera from patients or PDA mice were diluted 1:2. Titration of IL-6 and CA19.9 in serum was performed with the IL-6 ELISA Kit (D6050, R&D systems) and the CA19.9 ELISA Kit (ab108642, Abcam), respectively, as instructed by the suppliers.

RNA extraction and qRT-PCR

RNA was extracted from cells with a trizol-chloroform extraction method. The RNA was then precipitated by the addition of isopropanol, and the RNA pellet was washed with a solution containing 75% ethanol and 25% DEPC-treated water. After 5 minutes of centrifugation at 7500 rpm and 4°C, the RNA pellet was re-suspended with DEPC-treated water and warmed in a dry bath for 10 min. RNA dosage was performed with a spectrophotometer (Epoch, Biotek).

cDNA was obtained after the reverse transcription of 1µg of total RNA using the GoSCRIPT Tm Reverse Transcription system (Promega) according to the manufacturer's procedure. Specific genes were amplified from this cDNA by real time PCR with the GoTAQ qPCR Master Mix kit (Promega), using an Mx3000P Stratagene system. Relative expression was calculated as a ratio of the particular gene expression to a housekeeping gene expression (TBP). Different primers were used: human LIF; murine LIF; human p21; human POU3F2; human S100; and human/murine TBP.

Western blotting

Protein extracts from sNF96.2 cells, different stromal cells (FHN, RAW, HMC-1) or cancer cells (PANC-1, MIAPACA-2, BXPC-3, HN-14, B-TIM TUM, J-IPC, L-IPC, HN-14)

were prepared with RIPA buffer (Hepes 50mM, Nacl 150mM, EDTA 1mM, EGTA 1mM, 10% glycerol, 1% TritonX-100, 10µM ZnCl2 with protease inhibitors cocktail). Proteins were titrated using the Bradford Protein Assay Reagent (Biorad). Proteins were separated on gels by electrophoresis: protein extracts (20µg) were run on 12% NuPAGE Novex Tris-glycine Mini Gels and electrotransferred onto nitrocellulose membranes using an electrophoretic transfer system (Invitrogen). A classical procedure was then used as reported earlier (12). The primary antibodies used were: anti-LIF antibody (Santa-Cruz, Clone N18, sc-1336, 1/200), anti-S-100 Protein (Millipore, clone 15E2E2, MAB079-1, 1/1000), anti-Pou3F2 (Abgent, clone AP16803C, AP16803C, 1/1000), anti-P21 Waf1/Cip1 (Cell Signaling, DCS60, 2946S, 1/1000), anti-pAKT (Cell Signaling, clone D9E, 4060S, 1/1000), anti-STAT3 (Cell Signaling, clone 79D7, 4904S, 1/1000), anti-p-STAT3 (Cell Signaling, clone D3A7, 9145S, 1/1000), and anti-β-actin (Sigma-Aldrich, clone AC-74, A2228, 1/2000).

Concerning in vivo experiments, protein extracts of pancreas from PDA or healthy mouse were obtained after organ crushing and preparation with RIPA-2 buffer (Nacl 150mM, Tris-base pH8 20mM, 1% NP40, EDTA 5mM with protease inhibitors cocktail). The quantity of protein run was 20µg, and the following western blot steps were the same as with *in vitro* samples.

Immunofluorescence

Immunofluorescence experiments were performed either on cultured cells or on murine pancreas samples previously fixed in paraformaldehyde 4%, paraffin embedded and cut. Concerning PDA patients, immunofluorescence was performed on tissue microarray slides. The antibodies used were: anti-Pou3F2 (Abgent, clone AP16803C, AP16803C, 1/100), anti-LIF (Santa-Cruz, Clone N18, sc-1336, 1/50), and anti-S100 (Millipore, clone 15E2E2, MAB079-1, 1/100). Additional primaries antibodies were used to detect CD68 (Abcam, Ab845, 1/50), cd117/c-kit (Abcam, Ab5505, 1/100), α-SMA (Sigma-Aldrich, A2547, 1/200), cytokeratin 19 (Dako, M-0888, 1/50), marking respectively macrophages, mast cells, fibroblasts and epithelial pancreatic cancer cells. Primary antibody labeling was followed by Alexa Fluor (488nm or 568nm)-conjugated secondary antibody staining (Invitrogen, 1/500). Nucleic contrast was achieved with DAPI staining and slides were observed using a Nikon eclipse 90i fluorescence microscope. Image quantification was performed using the ImageJ software.

Immunohistochemistry

Slides from human or mouse paraffin embedded samples were processed for immunohistochemistry (IHC) using several primary antibodies: anti-Pou3f2 (Abgent, clone

AP16803C, AP16803C, 1/100), anti-LIF (Santa-Cruz, Clone N18, sc-1336, 1/50), anti-LIFR (Santa Cruz, clone C-19, sc-659, 1/10), anti-gp130 (Santa Cruz, clone E-8, sc-376280, 1/50), anti-S100 (Millipore, clone 15E2E2, MAB079-1, 1/100) and anti-Neurofilament 200 (NF200) (Sigma-Aldrich, clone N52, N0142, 1/400). Primary antibody labeling was followed by biotin conjugated secondary antibody staining. HRP-streptavidin and then DAB reagent were added for protein detection. Finally, Mayer's hematoxylin dye was used to color tissues.

Sensory neurons extraction from dorsal root ganglia (DRG)

To extract sensory neurons from DRG, six-week-old CD1 mice were anesthetized and then injected intracardially with Hank's Balanced Salt Solution (HBSS) (Life technologies) supplemented with HEPES 4.76 g/l, D-glucose 1,8 g/l, which allowed for neuron protection and blood removal during dissection. Then neurons present in the DRG were released by a collagenase/dispase (Liberase, n°05401160001, Roche) treatment supplemented with Cacl2 1M and dissociated by several passages through a 26G needle. "Next, neurons were counted and dispatched in 96-well plates (5000 neurons per well), and cultivated overnight in a neurobasal medium (Life Technologies) containing NGF (10ng/ml), GDNF (2ng/ml), B27 (0.02µg/µl), Glutamine (2mM), and Penicillin-Streptomycin (0.01µg/µl). For in vitro assays, neurons were plated in poly-L-Lysine (Sigma-Aldrich) and laminin (Invitrogen) pre-coated wells. The next day, medium was removed and replaced with fresh experimental neurobasal media without NGF and GDNF, and containing or not LIF (50ng/ml).

Results

Members of the gp130 "ligand/receptor" family are overexpressed in human and murine PDA

We previously showed that the stromal compartment can, through its secretory ability, impact nerve system reorganization within PDA tumors (12). Regarding recent studies revealing a role of inflammatory processes in PANR (11,22), we hypothesized that some genes/pathways, involved in the regulation of inflammatory processes, may be upregulated in the stromal compartment of pancreatic cancer and could impact PANR. Using two sets of RNA microarray analysis previously published by our group (Gene Expression Omnibus (GEO): GSE50570 for human PDA, (12), and GSE61412 for mouse PDA, (27)), we revealed that numerous members of the gp130 "ligand/receptor" family, with some already associated with neuropathic disorders or regulation of the nervous system (Table 1, column c), were upregulated in the PDA stromal compartment (Table 1, column d). Interestingly, we found that LIF (Leukemia Inhibitory Factor) was overexpressed both in stromal compartment from

human PDA (Table 1, column d) and at late stage in spontaneous pancreatic cancer mouse model (pdx1-cre/$Kras^{G12D}$/$Ink4A^{fl/fl}$) (Table 1, column g).

Gene name[a]	**Symbol[b]**	**Neural Association (+/-)[c]**	**Human[d]**	**Mice-4w[e]**	**Mice-6w[f]**	**Mice-9w[g]**
Interleukin 27 receptor, alpha	IL27RA	-	1.79	2.03	1.38	1.25
Leukemia inhibitor factor receptor (LIFR)	LIFR	+	1.76	-1.44	-1.07	-2.85
Oncostatin-M	OSM	+	1.69	1.26	1.22	2.17
Interleukin 6 signal transducer (gp130)	IL6ST	+	1.68	1.35	1.24	1.51
Leukemia inhibitor factor (LIF)	LIF	+	1.64	1.45	1.26	3.08
Interleukin 11	IL11	+	1.44	1.22	1.12	2.65
Cardiotrophin-like cytokine	CLC	+	1.34	1.55	1.57	5.27
Interleukin 11 receptor, alpha	IL11Rα	+	1.20	-1.09	1.02	-1.53
PRKR interacting protein 1 (IL11 inducible)	PRKRIP1	+	1.10	-1.03	1.01	1.22
Oncostatin-M receptor	OSMR	+	1.06	2.25	2.26	7.07
Interleukin 6 receptor	IL6R	+	1.06	-1.04	1.17	-1.23
Ciliary neurotrophic factor receptor	CNTF	+	-1.03	1.15	1.00	1.04

Interleukin 27	IL27	-	-1.75	-1.01	-1.15	-1.33

Table 1. Identification of gp130 family genes in human and murine pancreatic tumors. mRNA fold change of gp130 family genes in transcriptomic analysis of human (n=4) and mouse (n=9) pancreatic samples. (a) gene name; (b) gene symbol; (c) genes associated to nervous system; (d) Fold change of mRNA level in stromal versus tumor cell compartment from human PDA samples; (e-g) Fold change of mRNA level in spontaneous PDA versus healthy pancreas from mouse samples; (e) Four-week-old mice (early mPanINs); (f) Six-week-old mice (intermediate stage); (g) Nine-week-old mice (late PDA).

While the role of the LIF-gp130 pathway is well defined within nervous system regulation and inflammation (28-30), its implication in pancreatic tumorigenesis is poorly understood (31). We first analyzed the expression of LIF in human PDA samples and revealed that LIF expression was increased in PDA samples in contrast to its almost complete absence in healthy pancreas (Fig. 1A and B). Interestingly, regarding the hypothetical role of LIF in neural remodeling, we observed that nerve fibers within human PDA samples commonly expressed the two LIF receptors, LIFR and gp130 (Table 2). Indeed, LIFR proteins were present in 8 out of 12 nerves analyzed within PDA tumors with a mean expression of 9.7% inside nerves. In addition, gp130 was found in 4 out of 12 nerves but with a stronger mean expression (20%). These changes in LIF expression were confirmed in PDA mouse model with a strong increase in LIF mRNA (Fig. 1C) and protein (Fig. 1D and 1E) level in PDA samples compared to healthy pancreas. Altogether, these data reveal the presence of LIF and its receptors, LIFR and gp130, in PDA samples. Moreover, the expression patterns of LIFR and gp130 support the hypothesis of LIF implication in PDA associated neural remodeling.

Marker	Expression in nerve (%)	Number of nerves containing the marker (observed/total)	SD(+/-)	p-values	Tumor number (N)
LIF	1.9	9/12	0.2		
LIF-R	9.7	8/12	1.7	< 0.0001	8
gp130	19.5	4/12	2.6	0.0028	

Table2: Representative images of co-localization of LIF, LIFR or gp130 with neurofilament on human PDA. The table indicates the percentage of expression of the markers in nerves, and the fraction of nerves containing them. SD (+/-); p-values compared LIF-R or gp130 expression in nerves compared to LIF.

In PDA, secretion of LIF is mediated by the stromal compartment in vitro and in vivo

Regarding above data, we next sought to determine which cell types within PDA produced LIF. Using TMA of various human PDA samples, we observed that few epithelial cancer cells (cytokeratin-19) expressed LIF whereas higher percentages of macrophages (CD68), CAFs (αSMA) and mast cells (CD117) were labeled with LIF staining (Table 3).Such analysis on 8 different human PDA showed that only 6% of cancer cells expressed LIF whereas mast cells, macrophages and CAF expressed LIF at 21%, 34% and 47,5% respectively. This was confirmed by measurement of LIF mRNA expression in vitro, where fibroblasts (FHN) expressed higher amount of LIF mRNA than macrophages (RAW) or mast cells (HMC-1) (Fig. 2A). Interestingly, when co-cultured with RAW or HMC-1 or RAW+HMC-1, the fibroblasts showed an increased LIF mRNA expression (Fig. 2A). This level of LIF mRNA expression is similar with the one observed in primary CAFs from PDA patients (Fig. 7). This data suggests, as shown previously (11), that FHN co-cultivated with RAW reach a similar threshold of activation than primary CAFs.

Comparison of LIF mRNA levels in fibroblasts co-cultured with macrophages versus various established (PANC-1 and MIAPaCa-2) or primary PDA tumor cell lines (PDA≠1 to 4) revealed that stromal cells express the highest amount of LIF mRNA (Fig. 2B). Surprisingly, the increased level of LIF mRNA in PDA co-cultured stromal cells did not result in higher LIF protein levels compared to single stromal or tumoral cell cultures (Fig. 2C and D). Considering this discrepancy between mRNA production and intracellular protein levels, we hypothesized that there was a change either in translation machinery, in LIF degradation or in LIF secretion. As LIF is referenced as a secreted cytokine, we measured by ELISA the amount of LIF secreted in media and observed a higher LIF concentration in media from fibroblasts co-cultured with macrophages compared to other stromal cell cultures (Fig. 2E and Fig. 8A). Interestingly, whereas amount of LIF secreted by CAF was higher than by FHN, we observed that amounts of LIF secreted were not different between CAF and FHN when co-cultured with RAW. Importantly, LIF was either undetectable or present in small amount in media from various tumor cells (Fig. 2F). Altogether, these data revealed that while numerous cell types within PDA potentially express LIF, the ability to secrete it seems restricted to the stromal compartment and in particular mostly to activated fibroblasts, a major cell component of PDA microenvironment that we have recently linked to PANR (12).

Cell Marker	LIF co-staining (%)	SD(+/-)	Tumor number (n)
Cytokeratin-19 (cancer cells)	6.8	1.7	8
CD117 (mast cells)	21.8	4.4	8
CD68 (macrophages)	34.4	7.9	8
α-SMA (CAF)	47.5	8.7	8

Table 3: Co-localization of LIF with Cytokeratin-19, CD117, CD68 or α-SMA on human PDA sections. The table indicates the percentage of LIF co-localization with these markers on human PDA (n=8).

LIF enhances the migratory capacity of peripheral nerve Schwann cells

We sought to determine if the presence of secreted LIF in PDA could modulate nerve cells' abilities, and therefore have an impact on PANR. We investigated the effect of stromal conditioned media (CM) with the highest LIF titer (FHN+RAW and FHN+RAW+HMC1) on the migratory ability of peripheral nerve Schwann cells and we observed a two-fold increase of Schwann cell migration after 4h (Fig. 3A and Fig. 8B). To assess whether LIF from those stromal CM was the mediator of the observed migratory improvement, we blocked LIFR and consequently impaired LIF signaling with a blocking LIFR antibody (Ab-LIFR) and observed that migration ability is restored to control level (Fig. 3B, upper panel). Similar results were obtained using CM from CAF+macrophages and inhibition of LIFR compared to the use of a control antibody (Fig. 3B, lower panel). The use of SC144 and AG490, two chemical inhibitors which block gp130 (the co-receptor of LIF) or JAK2 signaling, the specific pathway activated after gp130/LIFR induction (32,33), respectively, confirmed previous data (Fig. 3C). Finally, LIF's specific ability to enhance the migration of peripheral nerve Schwann cells was confirmed with human recombinant LIF protein at a dose of 50ng/ml, determined as the lowest dose inducing the higher migration improvement (Fig. 3D). Such induced migration ability with LIF recombinant protein was inhibited using LIFR blocking antibody at the lower dose of 4µg/ml (Fig. 3E, upper and lower panel) but also using AG490 and SC144 (Fig. 3F).

Regarding intracellular signaling induced by LIF stimulation through its receptors, LIFR and gp130, STAT3 and AKT are two of the main pathways known to be induced (34). First, we confirmed that in SNF96.2 cells LIF can trigger STAT3 and AKT

phosphorylation/activation (Fig. 9A and 9B). We further confirmed that such signaling activation is mediated by LIF receptors, LIFR and gp130, as AG490 or LIFR blocking antibody were able to inhibit STAT3 and AKT phosphorylation (Fig. 9C). As reported in Figure 3G, stromal cells CM could induce STAT3 or AKT phosphorylation/activation. However, the use of AG490 or LIFR blocking antibody inhibited stromal cells CM effects on intra-cellular signaling suggesting that the CM-derived LIF could no longer activate LIFR/gp130 signaling. Altogether, our results revealed that LIF from stromal cell conditioned media is able to induce Schwann cell migration through LIFR/gp130 signaling then STAT3/AKT phosphorylation/activation.

LIF inhibits Schwann cell proliferation

We next examined the effects of stromal cell CM on Schwann cell proliferation and revealed that 48h of incubation with the highest LIF-titrated CM (FHN+RAW or FHN+RAW+HMC-1) decreased cell proliferation by 16% (Fig. 4A) without affecting cell survival (Fig. 10A). We validated that this decreased cell proliferation was due to the presence of LIF by adding the LIFR blocking antibody to the CM, which restored cell proliferation to the control level (Fig. 4B). We obtained similar results using LIF recombinant protein with a cell growth reduction of about 17% (Fig. 4C) without modification of cell survival (Fig. 10B).

In agreement with previous report linking JAK/STAT3 pathway activation and cell growth arrest (35), we observed an increase in p21 mRNA level by 24 hours post-LIF treatment (Fig. 4D) and an increase in p21 protein level by 36 and 48 hours (Fig. 4E). Interestingly, p21 protein level is restored with AG490 or SC144 treatments on cells incubated with LIF recombinant protein (Fig. 4F) or with stromal cells CM (Fig. 4G). These data highlight the impact of LIF secreted by PDA stromal cells on the reduction of Schwann cell proliferation, which occurs concordantly with their enhanced migratory abilities.

LIF induces Schwann cell differentiation and neuronal plasticity

The inventors performed S100 and Pou3F2 staining on human PDA and Pou3F2 and S100 dual-staining in sNF96.2 cells incubated for 48 hours with control (sNF96.2) or stromal (FHN+RAW or FHN+RAW+HMC-1) conditioned media (CM), in the absence or presence of AG490 or SC144.

Interestingly, JAK/STAT3 pathway is known to induce cell differentiation, a crucial process for nerve cells involved in PANR. Thus, we analyzed Pou3F2 and S100, two independent markers of Schwann cell differentiation (36,37) that we found expressed in human PDA nerve fibers. Interestingly, we observed an induction of both markers in

Schwann cells after 48 hours of incubation with stromal cell CM (Fig. 5A, left panels). Such increased was lost when stromal cells CM was supplemented either with LIFR blocking antibody (Fig. 5A, right panels), AG490 or SC144. Moreover, we showed that incubation with LIF recombinant protein was able to induce Pou3F2 and S100 expression in Schwann cells at both mRNA (Fig. 5B) and protein levels (Fig. 5C). Besides its impact on Schwann cells, we wondered whether LIF may affect neuronal plasticity associated to PANR (38). As suspected, we found that recombinant LIF could induce neuronal plasticity with increased neurite outgrowth (Fig. 5D) and soma area (Fig. 5E). Those data reveal that LIF, secreted by PDA stromal cells, is able to induce Schwann cell differentiation and neuronal plasticity. In addition to data shown in previous parts, our study firmly support the potent impact of LIF in the neural remodeling observed in PDA tumors.

LIF titer in serum as a diagnostic and prognostic biomarker for PDA patients

To definitively assess if LIF is an inductor of PANR in vivo, we first analyzed LIF titer in sera from PDA bearing mice compared to LIF titer in sera from healthy mice and mice developing acute or chronic pancreatitis. Interestingly, not only we found a significant increase in LIF titer sera in PDA-bearing mice compared to control or benign pancreatic diseases (Fig. 6A) but we found that LIF titer in sera from PDA-bearing mice is positively correlated with intra-tumoral nerve density ($R2=0.82$, $n=12$, Fig. 6B). Finally, we assessed in vivo if LIF was directly influencing intra-tumoral nerve density by using a LIF neutralizing antibody in mice developing PDA. As shown in figure 6C, control mice (treated with a control antibody) displaying a low level of LIF in serum (<124pg/ml) exhibit few intra-tumoral nerves while control mice displaying a higher level of LIF in serum (>124pg/ml) showed a significant increase in the intra-tumoral nerve density. Interestingly, mice treated with the LIF neutralizing antibody showed a significant reduction of intra-tumoral nerves in spite of the presence of a high LIF quantity in serum (>124pg/ml) (fig6F). Those data revealed that LIF is directly enhancing intra-tumoral nerve density in PDA and that LIF titration in serum could serve as a biomarker to predict PANR.

Using a cohort including human sera from healthy donors (N=61), PDA patients (N=142), or patients with chronic pancreatitis (N= 31) or benign pancreatic tumor (N=11) with cystic adenomas and IPMN (Intraductal Papillary Mucinous neoplams), we confirmed previous mice data (Fig. 6A) and showed that LIF titer was only increased in sera from PDA patients compared to other groups (Fig. 6D). Also we confirmed the positive correlation between LIF titer in sera from PDA patients and intra-PDA nerve density in human PDA ($R2=0.74$, $n=10$, Fig. 6E). Above data suggest that LIF titer in serum is associated to PANR

and could help in classifying PDA patients in terms of PANR grade. Moreover, when comparing patients with stage I–IV pancreatic cancer to merged healthy and chronic pancreatitis cohorts, the receiver operating characteristic (ROC) curves showed that adding LIF tittering to CA19.9 tittering improved significantly the area under the curve (AUC) from 0.901 for CA19.9 alone to 0.984 for CA19.9+LIF (Fig. 6F) with an associated improvement of specificity and a reduction of false-positive.

Altogether, these data support the use of LIF titer as a diagnostic marker for all stages of pancreatic cancer and as a biomarker to discriminate PANR level in PDA patients.

Discussion

Considering the grim mean survival rate among pancreatic cancer patients as well as the limited improvement of clinicians' arsenal over the last twenty years, it has become urgent to explore new therapeutic avenues that target PDA evolution as well as PDA-associated phenotypes. Among the latter, neuropathic pain and cachexia are major problems; management of these symptoms is fraught with difficulties, and globally, there exists no agreed upon standard care or treatment. Importantly, both symptoms are often the determining factors in deciding between patients' eligibility for chemotherapy or palliative care. Among fields to explore in order to improve drug accessibility and maintenance of treatment in PDA patients, deciphering mechanisms underlying PDA associated neural remodeling could yield promising results.

Although clinicians have for many years reported nervous system reorganization in cancers, and specifically in PDA, fundamental researchers have only recently realized its possible implications in PDA evolution and patient survival (8,13). It is now well acknowledged that infiltration of the tumor microenvironment by nerves, termed neoneurogenesis or axonogenesis, which occurs early in PDA development (11), plays an active role in cancer progression (39) and correlates with shortened survival, pain and local tumor recurrence (8). Although several studies have reported the ability of cancer cells to attract nerve fibers (40,41), very few have reported the impact of stromal cells in this process (12), especially in PDA where stromal cells compose the vast majority of the tumor cell mass. Therefore, our goal was to identify molecular targets from the PDA microenvironment that are involved in PANR, which may lead to the discovery of potent future adjuvant therapies that could prolong survival and reduce morbidity by blocking PANR. Here, we demonstrated for the first time that LIF, secreted by the PDA microenvironment, induced nerve cell migration and differentiation and thereby is positively correlated with PANR and axonogenesis (Fig. 6G). Concomitantly, we have revealed that LIF is a potent biomarker for

PDA, improving the overall significance of the classical tumor markers, CA19.9, and helps in determining PANR in PDA.

In this study, we considered knowledge associating tumor inflammation both with pancreatic cancer (42) and with the modulation of the nerve compartment (43,44) to reach our hypothesis that stromal-driven inflammatory genes/pathways could, additionally to their effects on tumor cells, impact the nerve compartment and in particular PANR. Thus, we revisited previous transcriptomic analysis (Gene Expression Omnibus (GEO): GSE50570 for human PDA, (12), and GSE61412 for mouse PDA, (27)) and highlighted numerous genes that code for molecules involved in gp130 signaling and were overexpressed in the PDA stromal compartment compared to PDA tumor cells.

Among, the identified gp130-related genes, we focused on LIF due to its major role in regulating the nervous system (45). Indeed, very little is known of its potent role in this context except a study suggesting that increased levels of LIF in PDA could impact the STAT3 pathway in cancer cells (31). In addition to confirming these results in both human and mouse PDA, our study has extended our knowledge on LIF from its expression pattern to its mode of secretion within PDA, revealing that although both tumor and stromal cells (CAFs, mast cells and macrophages) were able to express LIF, only stromal cells could secrete it. This striking and somewhat unexpected result reinforces the potent role of the stromal compartment in PANR but also raises questions about the role of this non-secreted LIF within PDA cancer cells. In our study we were interested in the effect of the stromal cell-secreted LIF in human PDA and found that infiltrating nerve fibers expressed LIFR and gp130 indicating a possible triggering of LIF signaling within these nerve cells.

We extended our in vivo data with in vitro experiments performing heterotypic co-cultures of stromal cells. We observed that co-cultures with macrophages drastically enhanced LIF secretion by fibroblasts, which is consistent with recent findings concerning LIF expression by activated fibroblasts (46). Here, we demonstrated that LIF is a strong modulator of nerve cell status, in terms of motility, proliferation and differentiation. Additionally, we confirmed, with blocking antibodies or chemical inhibitors of gp130, LIFR or the JAK/STAT3 signaling pathway, that all modulations observed on cell behaviors were dependent on LIF signaling. These results are particularly relevant from a therapeutic point of view: targeting LIF signaling through the inhibition of either LIF binding to its receptors or LIF-triggered signaling could, in addition to the reported effect on targeting cancer cells, have an impact on tumor progression via the inhibition of nerve infiltration.

However, while we had demonstrated that LIF could modulate nerve cell status in vitro and backed this up by revealing a plausible cellular expression of LIF, LIFR and gp130 in human PDA, we needed a correlation between the presence of LIF in PDA patients and PANR. Therefore, we measured LIF titer in serum from human or mouse PDA and correlated it with the nerve density in the corresponding PDA sample. In both models, we found a positive correlation between the amount of LIF in the serum and the intra-tumoral nerve density, supporting a link between LIF and PANR as well as revealing LIF as a valuable biomarker to determine PANR level in PDA. In addition to confirming our hypothesis, we observed that LIF titration in serum from PDA patients could have other uses. Indeed, as already reported for IL-6 and IL-11, cytokine serum levels are valuable diagnostic and prognostic tools (47-49) even if their low specificity restricted their use in clinic. Interestingly, LIF titration did not have this limitation as it improved the specificity of the existing test of reference; the tittering of CA19.9. Thus, the specificity given by LIF to distinguish PDA from other benign pancreatic diseases suggests that the combined detection of LIF and CA19.9 has greater usefulness than CA19.9 alone in the diagnosis of PDA.

Altogether, our results have potential therapeutic implications by providing a rationale for the use of LIF inhibitors in PDA, but also diagnostic implications by highlighting the usefulness of combining LIF and CA19.9 titration as a diagnostic and predictive marker. Indeed, our study is a proof-of-concept that the stroma impacts nervous system reorganization and thus PANR through the secretion of LIF. This secreted LIF (titrated in the serum), in addition to correlating with nerve density in PDA, exhibited a strong specificity with PDA tumors. While potentially useful in PDA detection, LIF titration should also be explored in a large panel of human cancers, especially those developing axonogenesis or perineural invasion such as prostate, colon and breast cancers. Further work will also be needed to determine the exact effect of LIF inhibitors in PDA, through the combined action on infiltrating nerves and on cancer cells.

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Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

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CLAIMS:

1. A LIF inhibitor for use in the treatment of PDA associated neural remodeling (PANR) in a subject in need thereof.

2. The LIF inhibitor for use according to claim 1 for reducing neuropathic pain in a subject in need thereof.

3. The LIF inhibitor for use according to any of claims 1 or 2 wherein said LIF inhibitor is selected from the group consisting of small organic molecule, polypeptide, aptamer, antibody, oligonucleotide or ribozyme.

4. The LIF inhibitor for use according to any of claims 1 or 2 wherein said LIF inhibitor is selected from the group consisting of LIF expression inhibitor, LIFR antagonist, LIFR expression inhibitor, gp130 antagonist, gp103 expression inhibitor, JAK1 inhibitor, JAK2 inhibitor and STAT3 antagonist.

5. The LIF inhibitor for use according to any of claims 1 or 2 wherein said LIF inhibitor is SC144 or AG490.

6. The LIF inhibitor for use according to any of claims 1 to 5 in combination with an anti-PDA treatment.

7. The LIF inhibitor for use according to claim 6, wherein said anti-PDA treatment is selected from the group consisting of gemcitabine, fluorouracil, FOLFIRINOX, nab-paclitaxel, inhibitors of PD-1, PD-1 ligand PD-L1, anti-CLA4 antibodies, EGFR inhibitors such as erlotinib, chemoradiotherapy, inhibitors of PARP, inhibitors of Sonic Hedgehog, gene therapy and radiotherapy.

8. A method of screening a candidate compound for use as a drug for treating PDA associated neural remodeling (PANR) in a subject in need thereof, wherein the method comprises the steps of:

   - providing a LIF, a LIFR, a gp130, providing a cell, tissue sample or organism expressing a LIF, a LIFR, a gp130,

- providing a candidate compound such as a small organic molecule, a polypeptide, an aptamer, an antibody or an intra-antibody,
- measuring the LIF activity,
- and selecting positively candidate compounds that inhibit LIF activity.

9. A method of identifying a subject having or at risk of having or developing PDA associated neural remodeling (PANR), comprising a step of measuring in a biological sample obtained from said subject the expression level of LIF.

10. The method of claim 9 which comprises a step consisting of comparing the expression level of LIF in the biological sample with a reference value, wherein detecting differential in the expression level of LIF between the biological sample and the reference value is indicative of subject having or at risk of having or developing PDA associated neural remodeling (PANR).

11. A method of treating PDA associated neural remodeling (PANR) in a subject in need thereof comprising the steps of:

(i) identifying a subject having or at risk of having or developing a PDA associated neural remodeling (PANR) by performing the method according to any claims 9 or 10, and

(ii) administering to said subject a LIF inhibitor when it is concluded that the subject has or is at risk of having PDA associated neural remodeling (PANR).

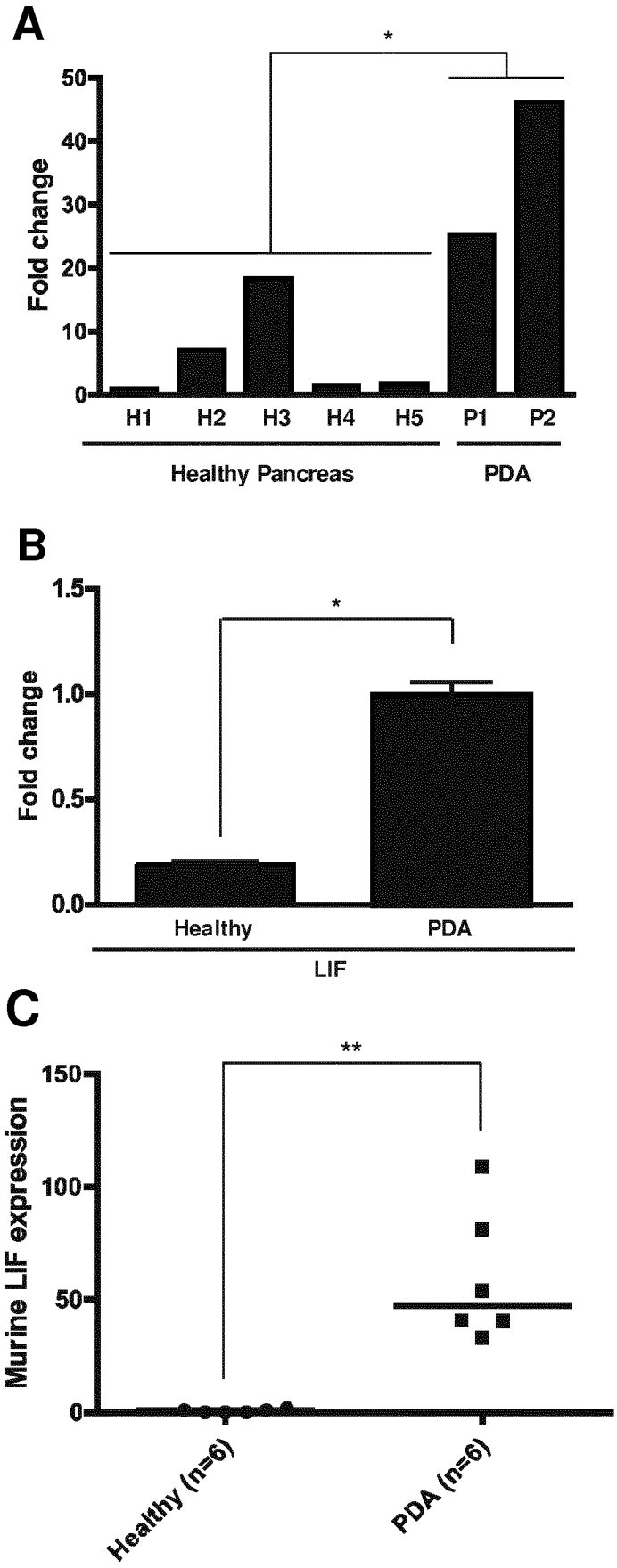


Figure 1 A, B and C

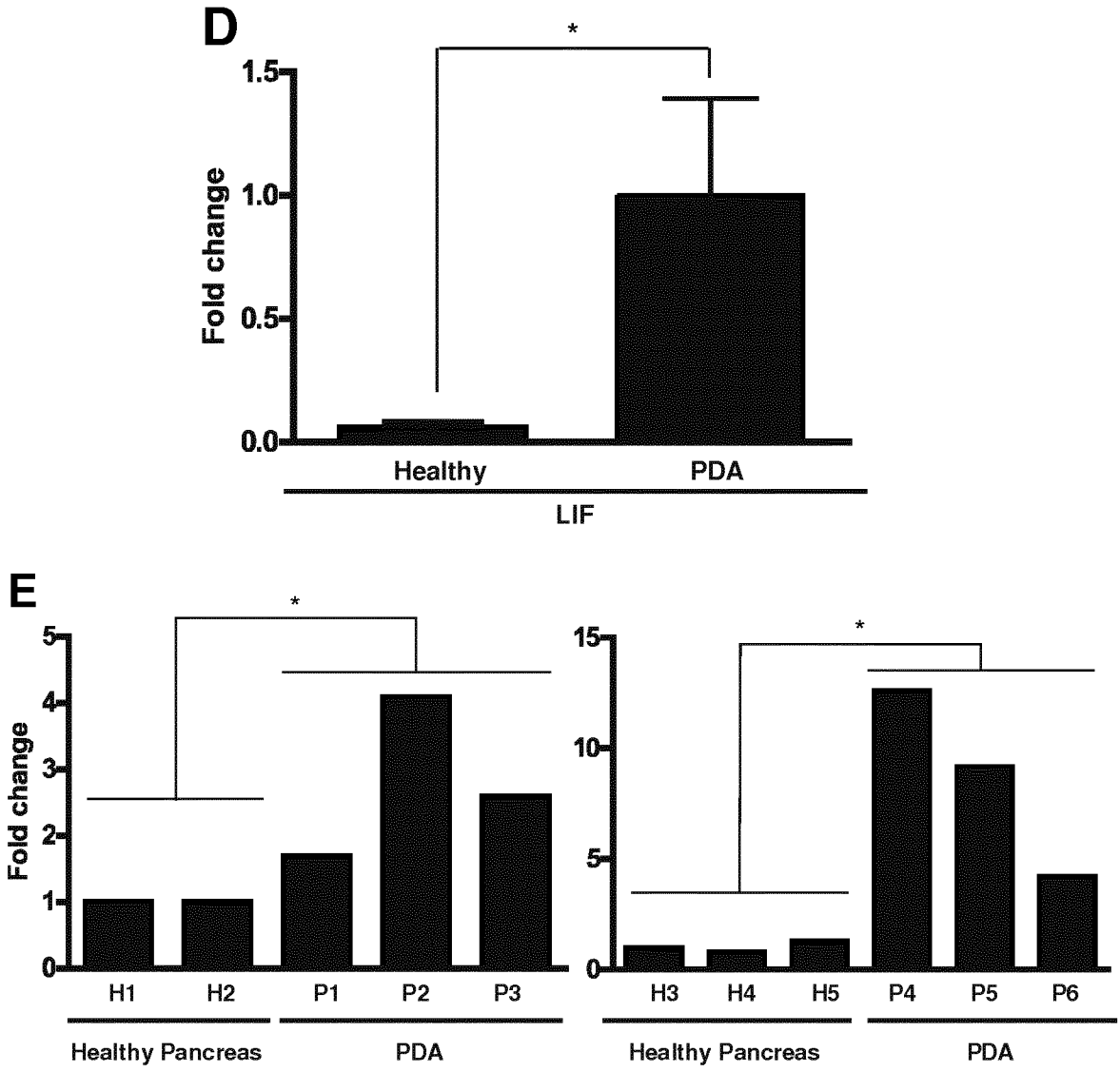


Figure 1 D and E

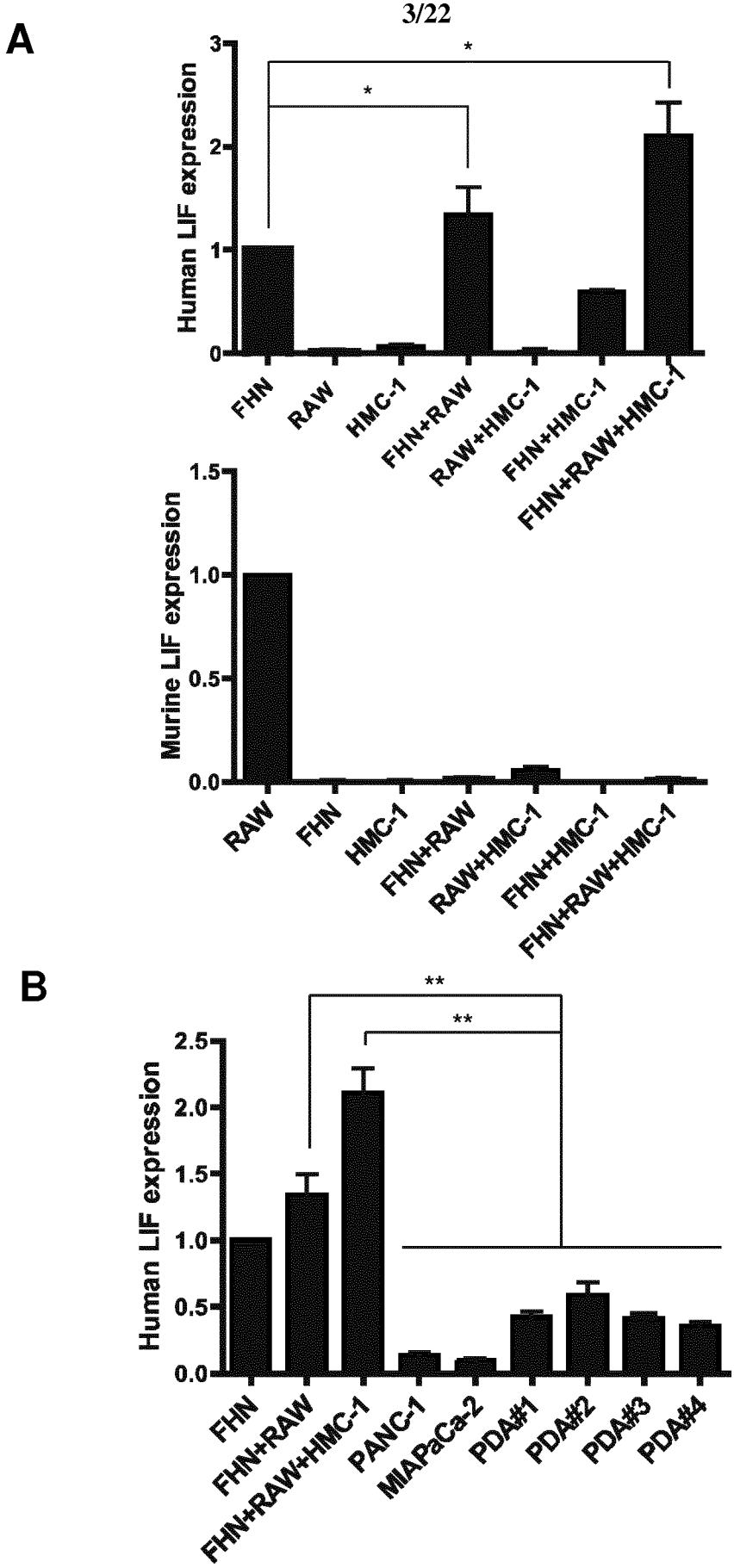


Figure 2 A and B

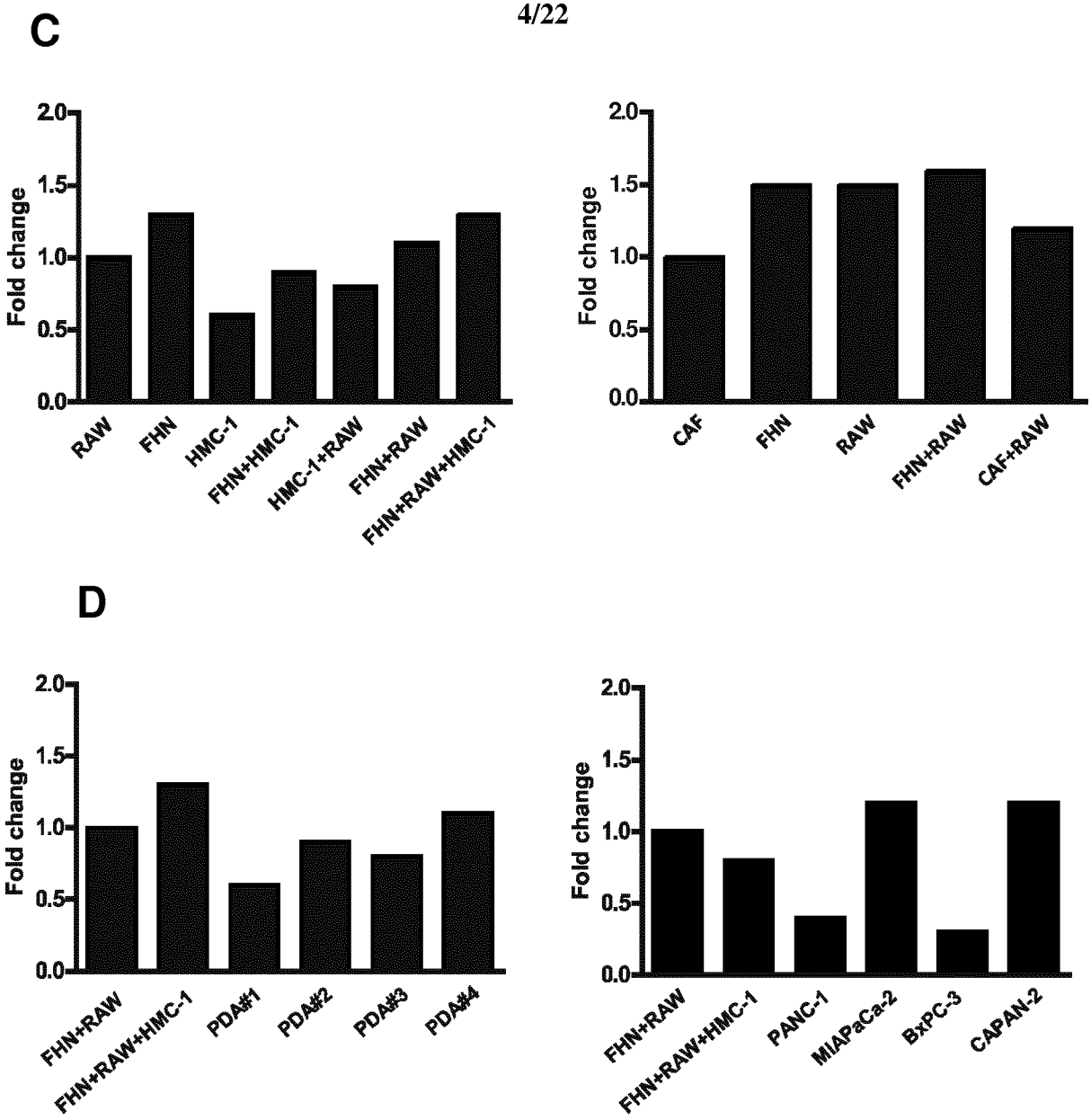
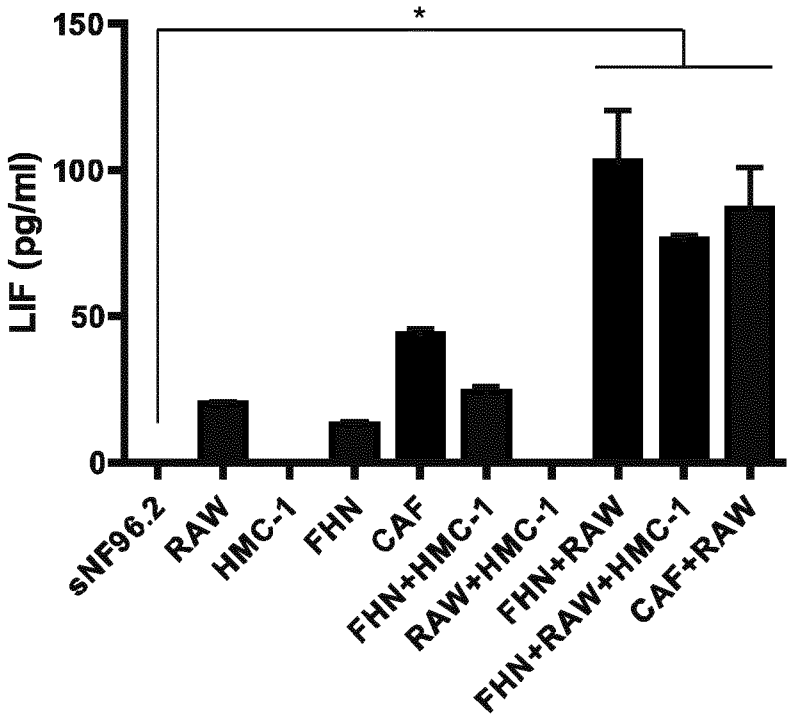


Figure 2 C and D

E



F

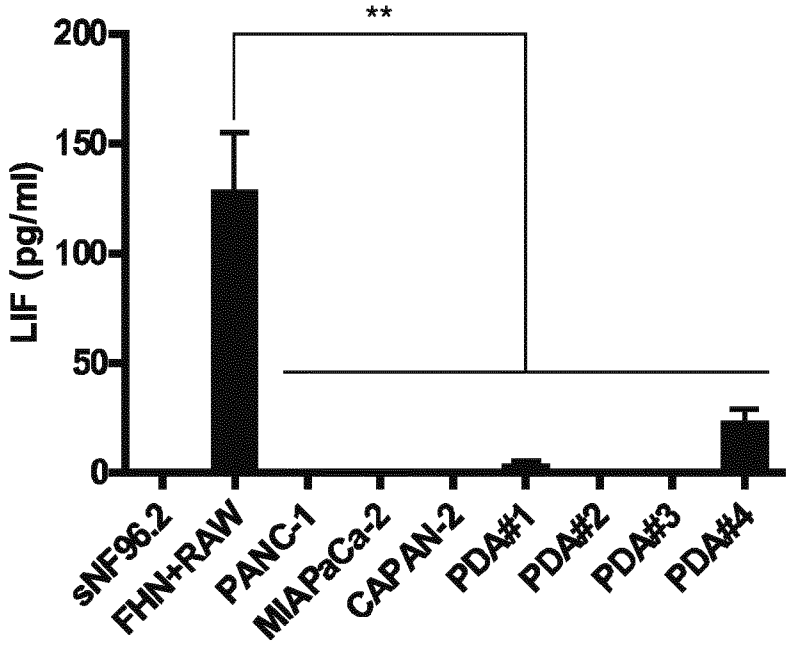


Figure 2 E and F

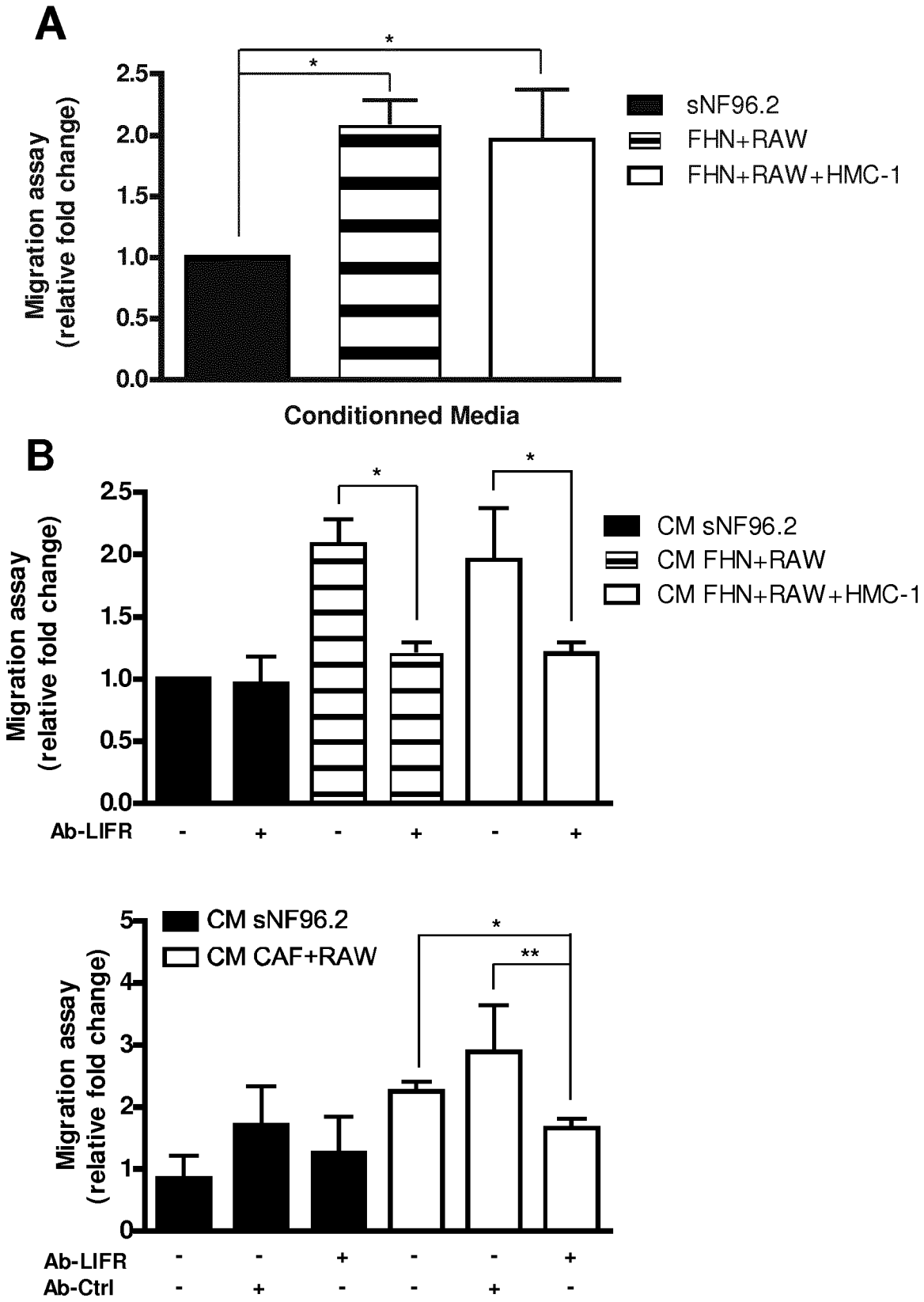


Figure 3 A and B

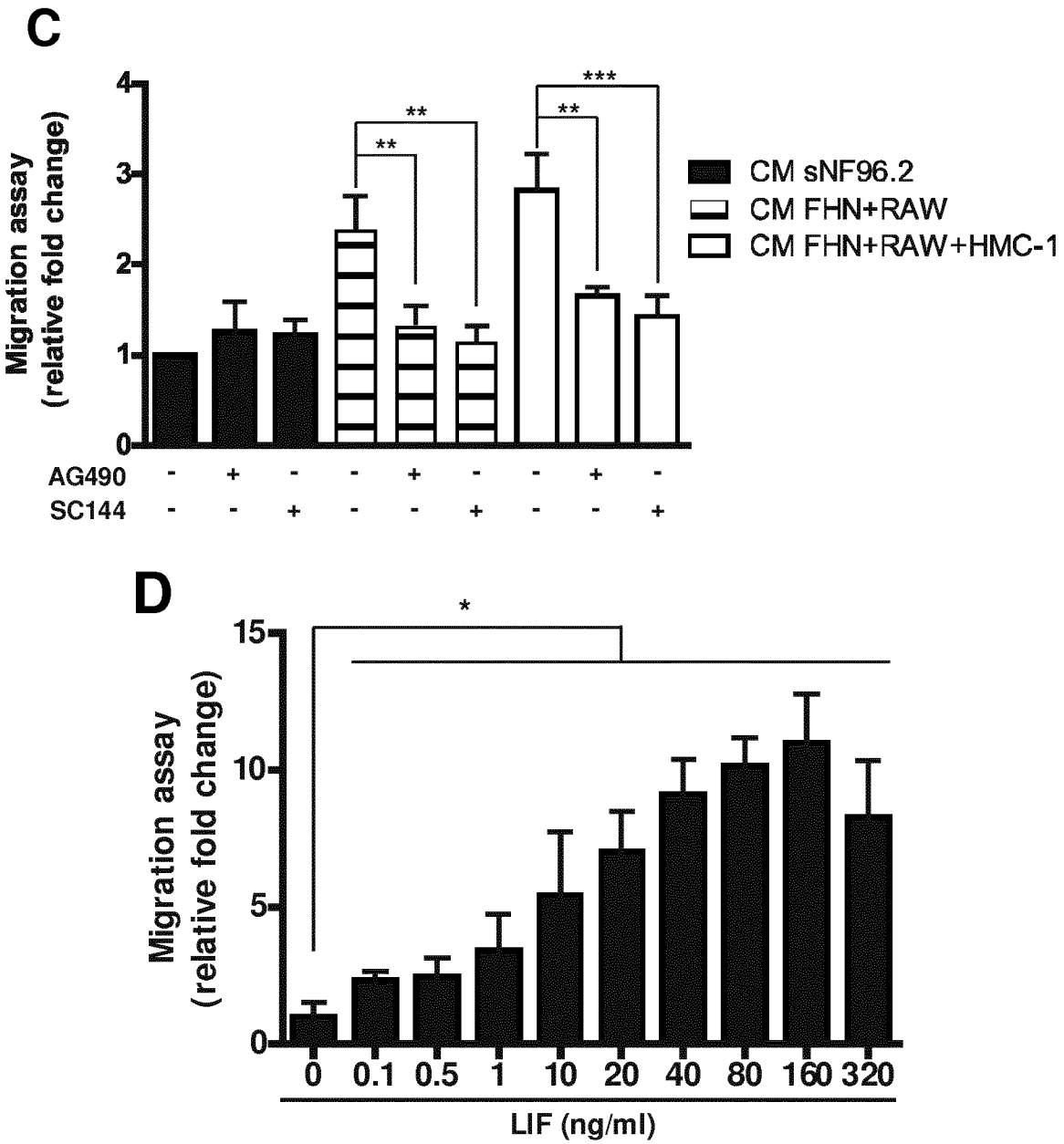


Figure 3 C and D

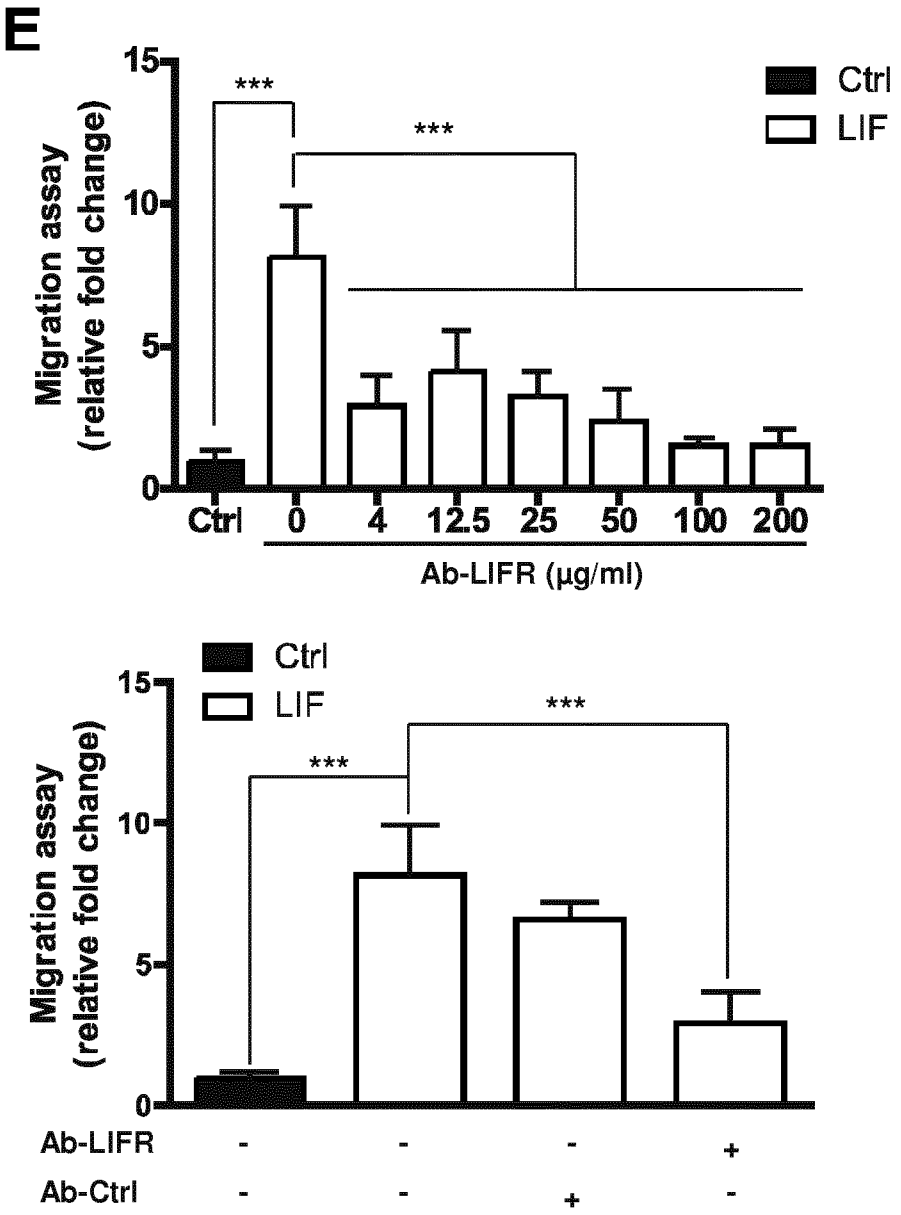

E
Migration assay (relative fold change)
15
10
5
0
***
***
Ctrl
LIF
Ctrl
0
4
12.5
25
50
100
200
Ab-LIFR (µg/ml)
Ctrl
LIF
***
***
Ab-LIFR - - - +
Ab-Ctrl - - + -

Figure 3E

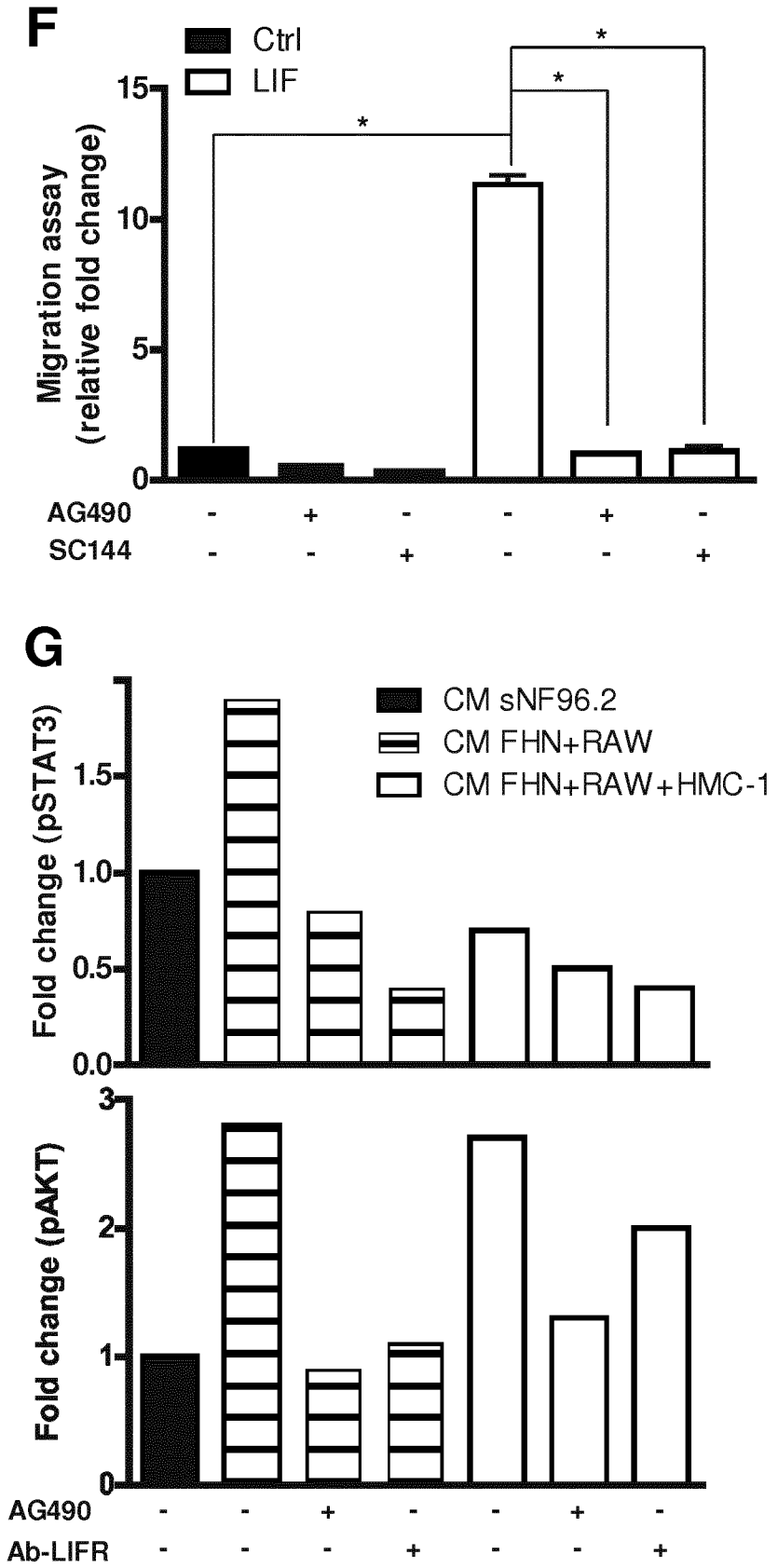


Figure 3 F and G

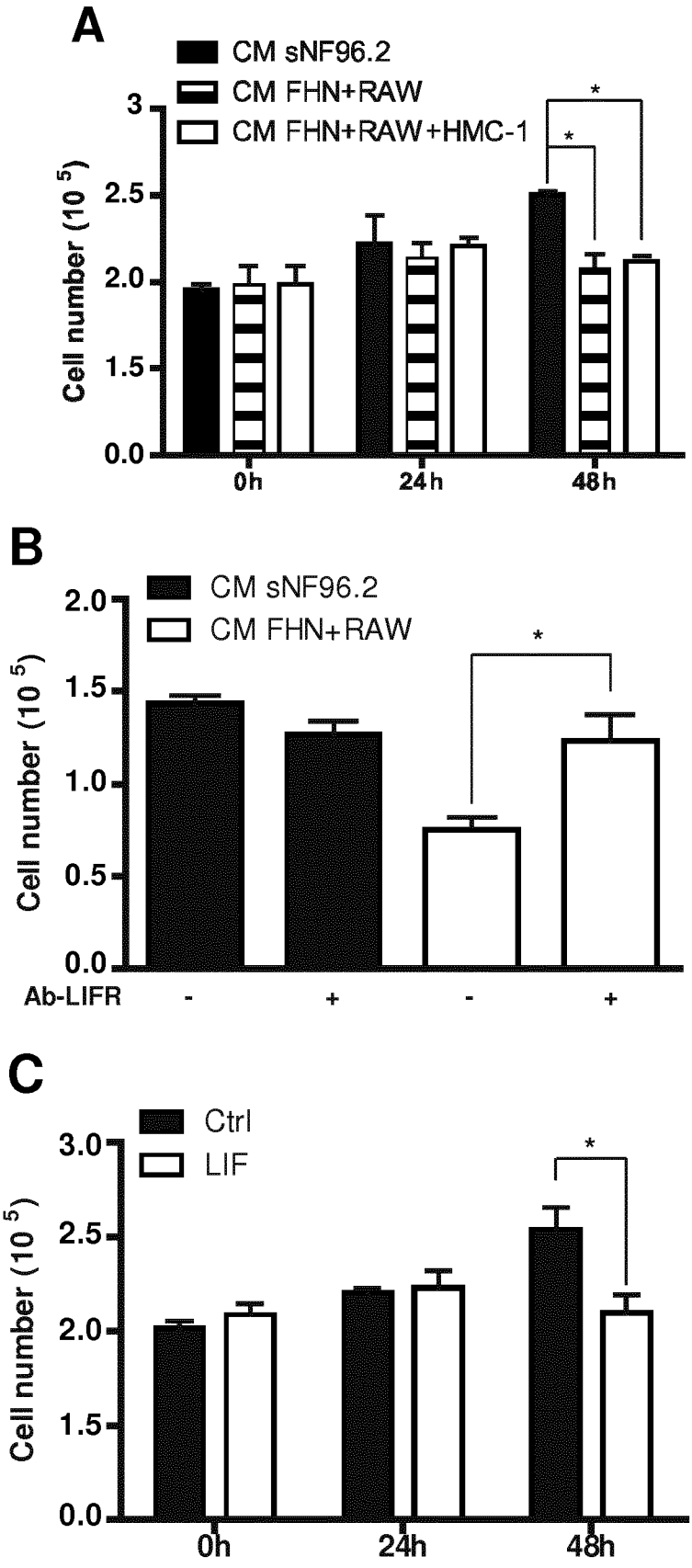


Figure 4 A, B and C

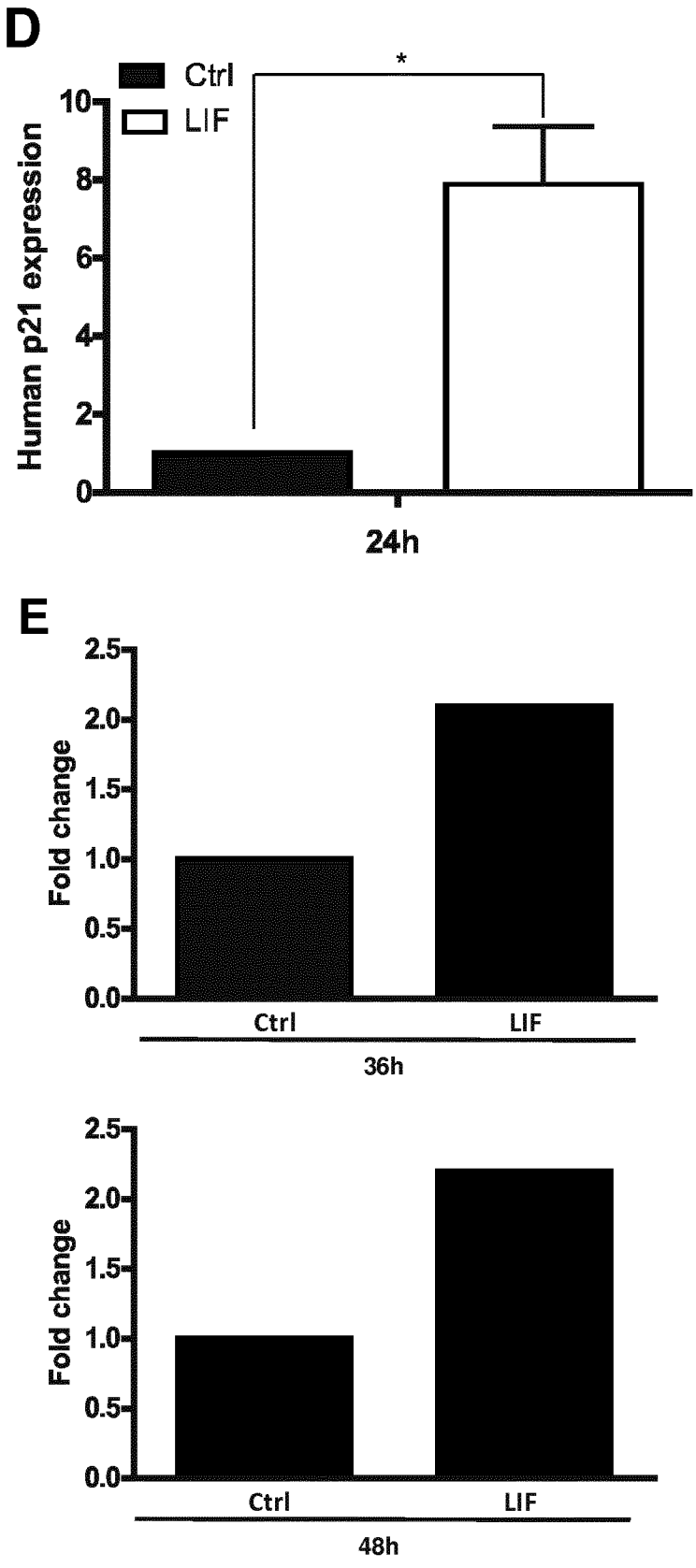


Figure 4 D and E

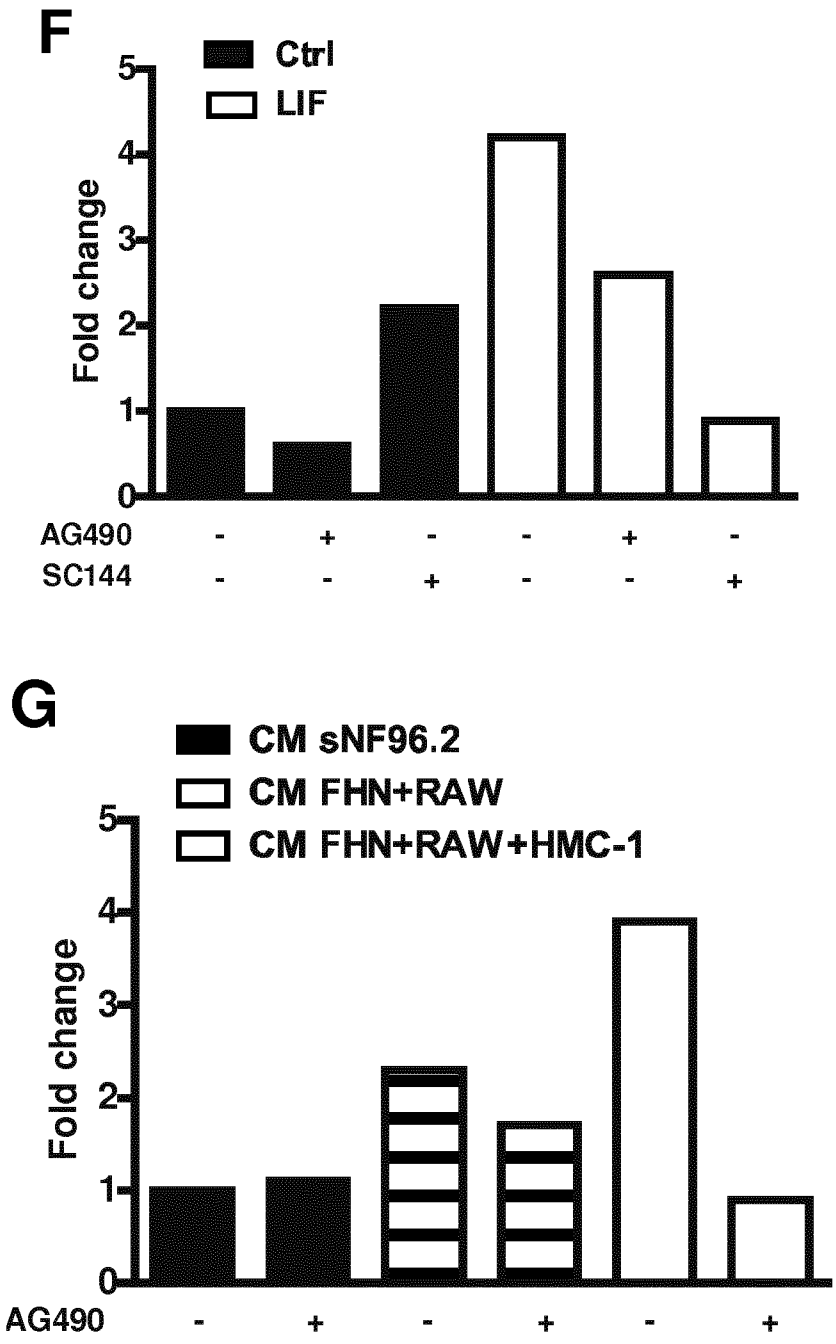


Figure 4 F and G

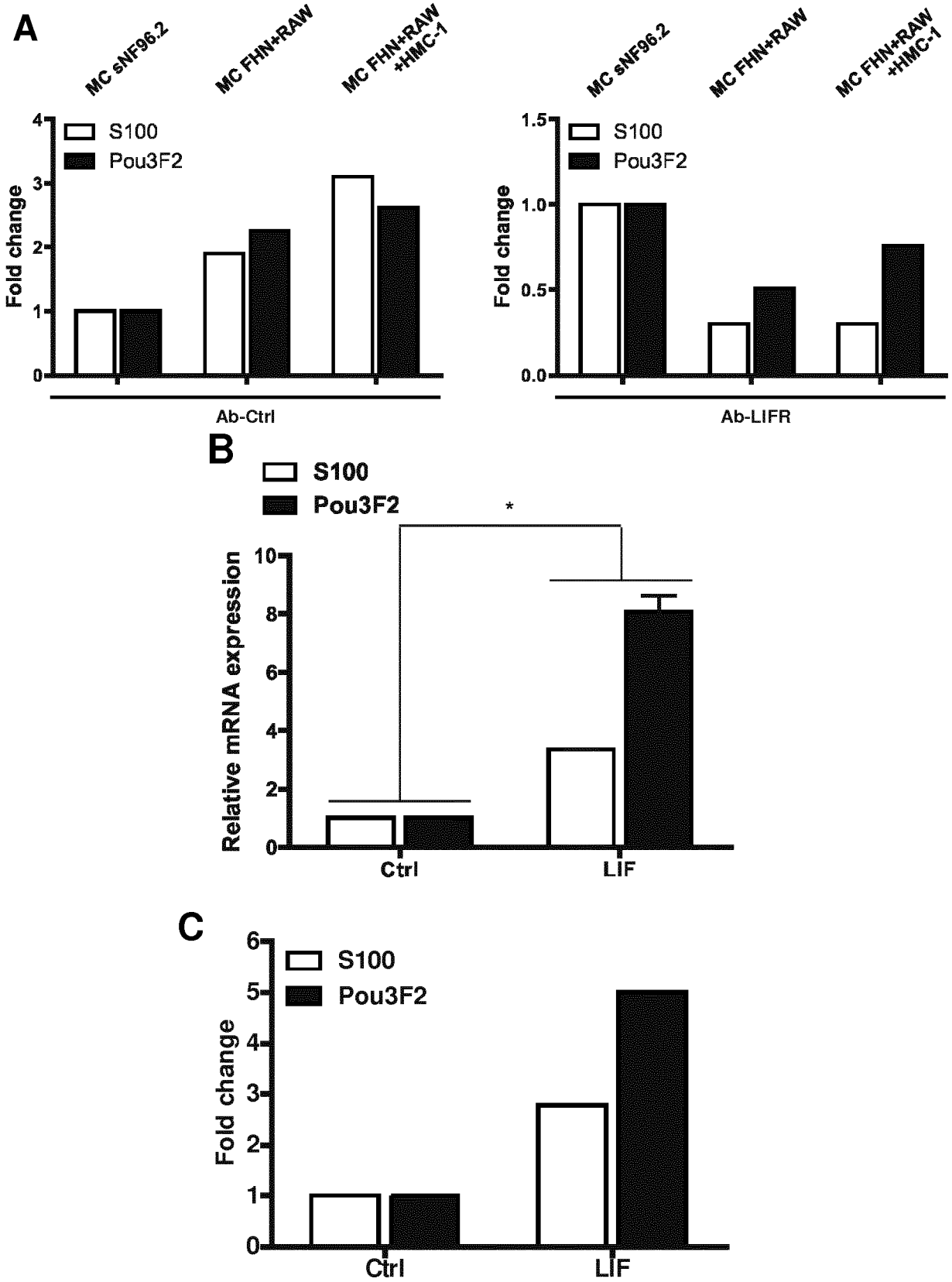


Figure 5 A, B and C

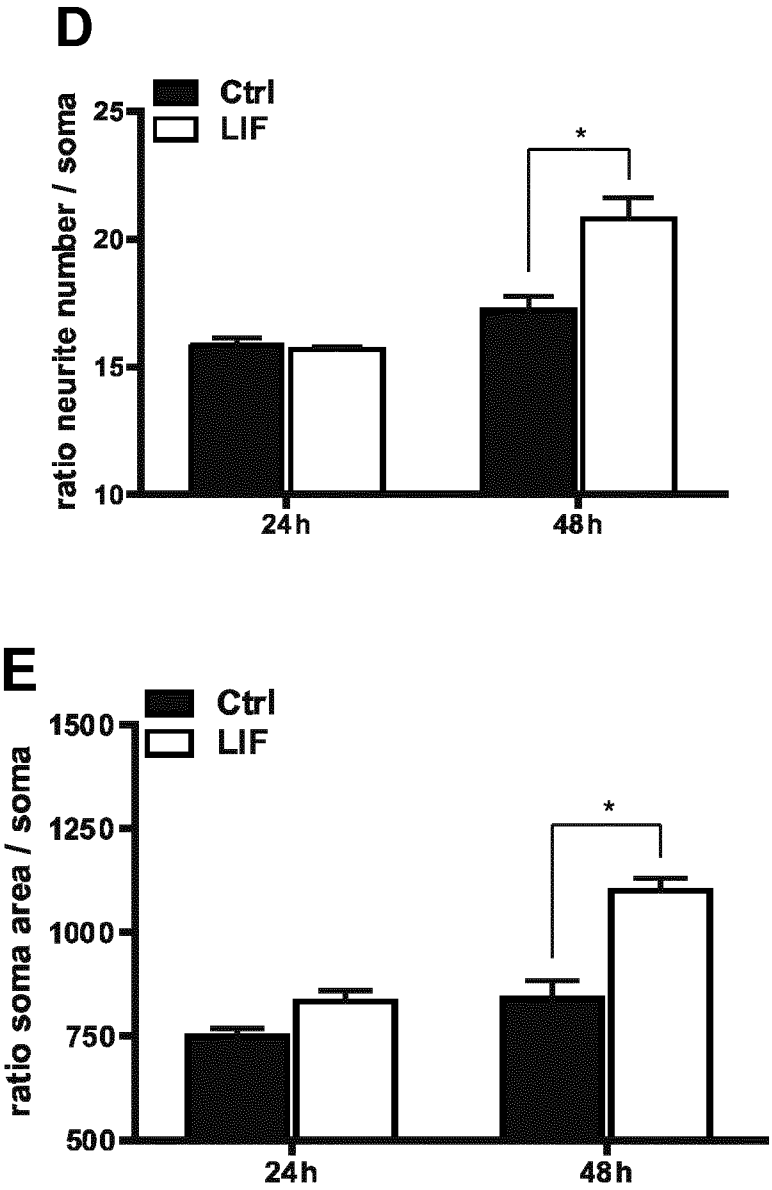


Figure 5 D and E

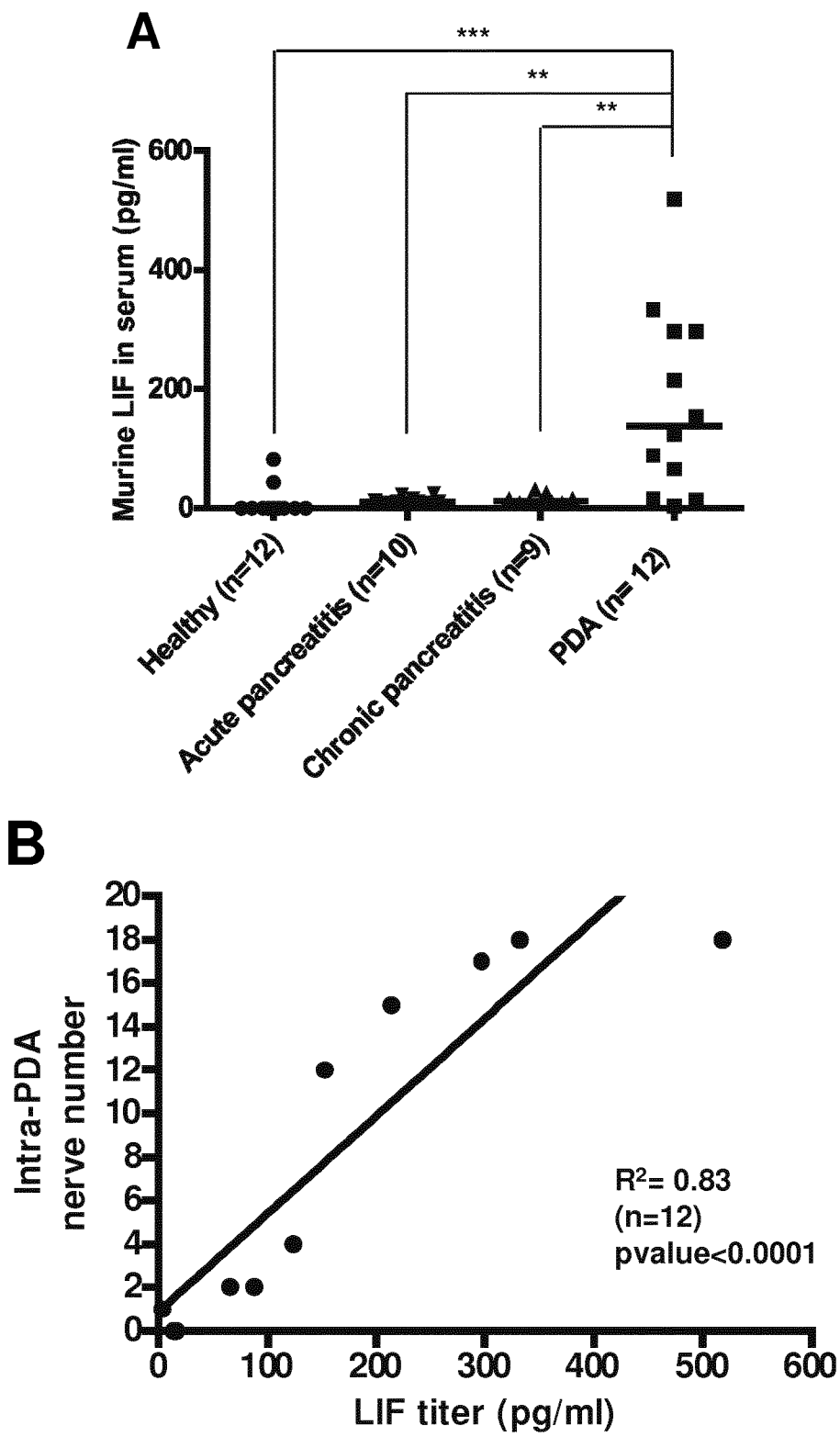
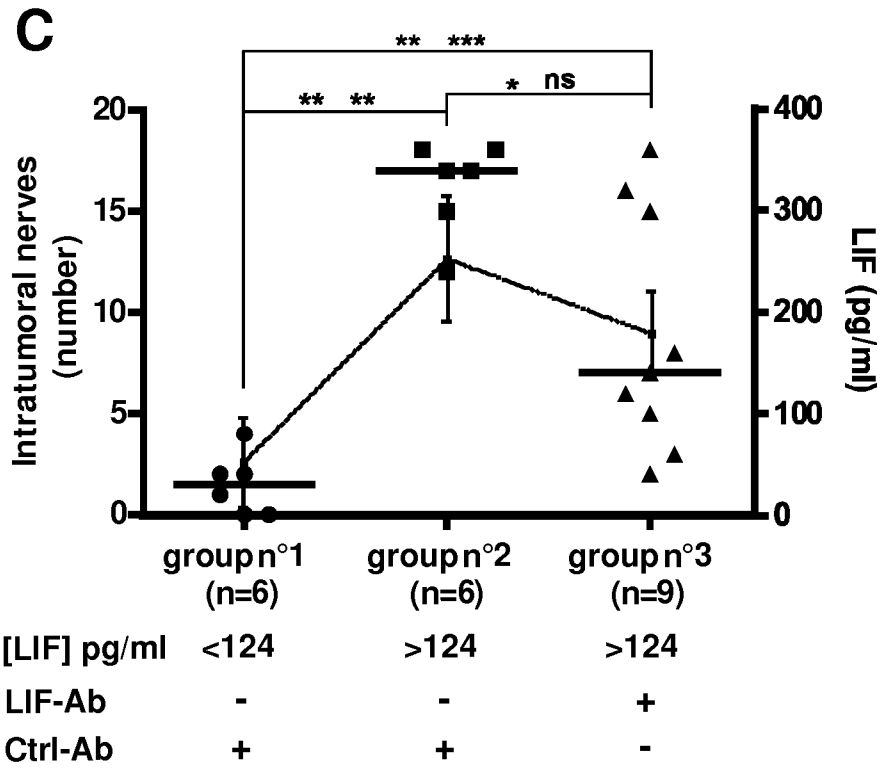


Figure 6 A and B



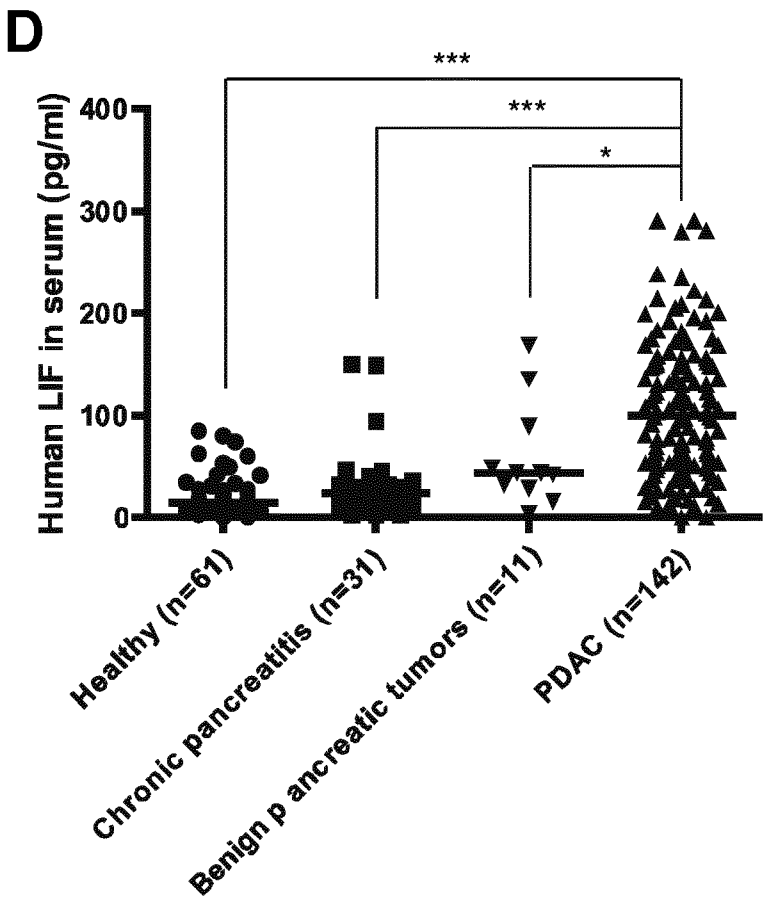


Figure 6 C and D

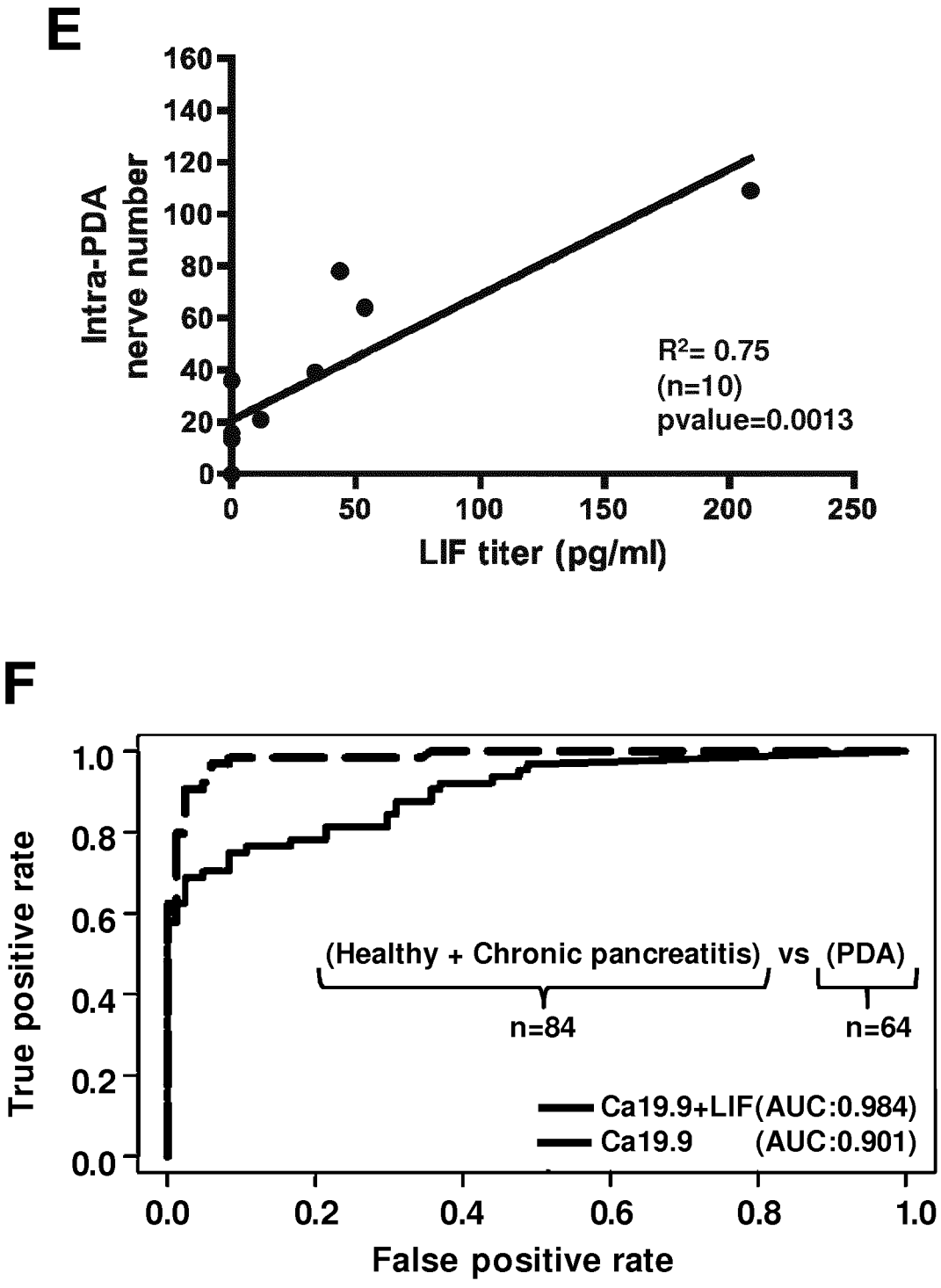
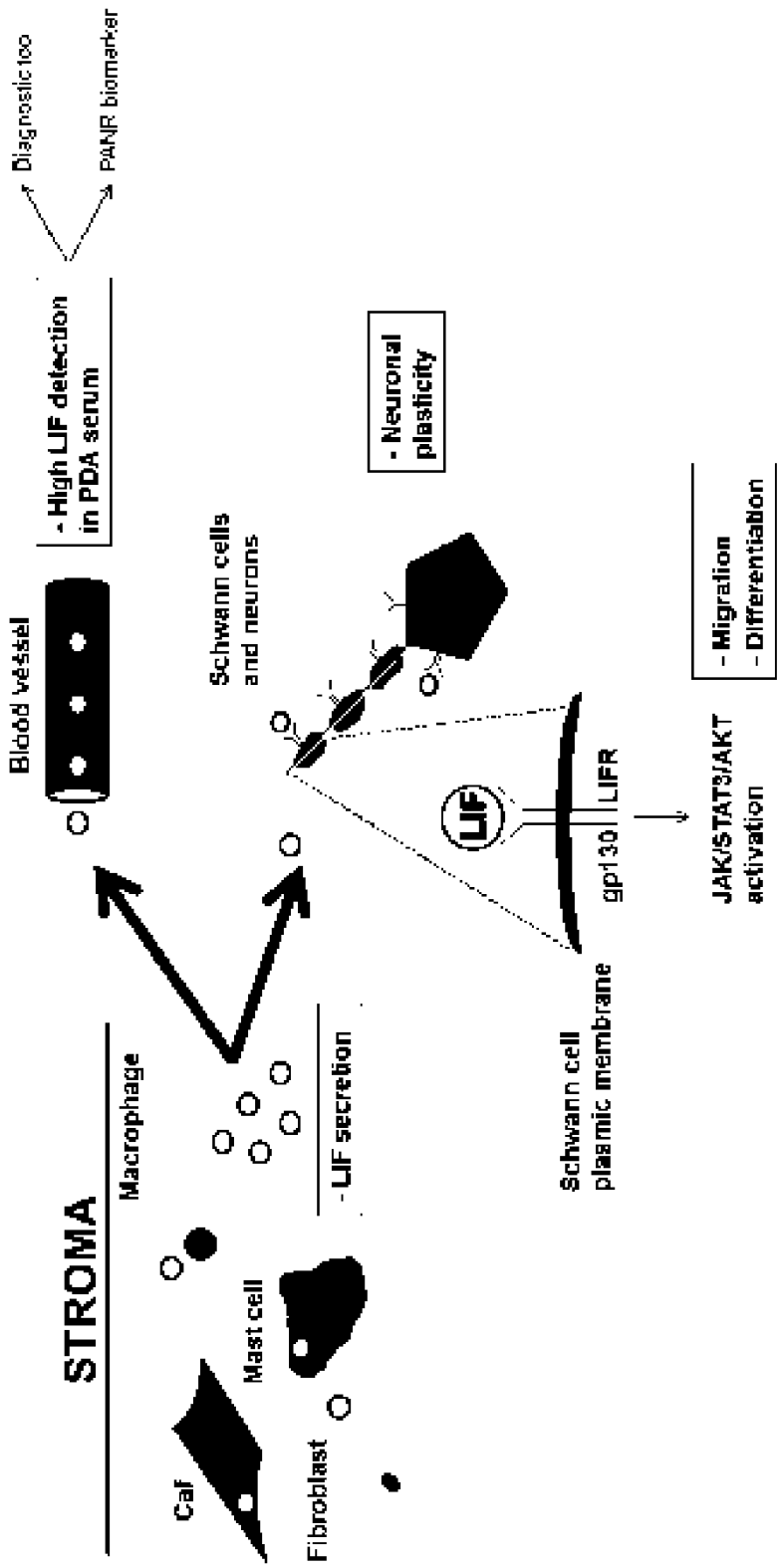


Figure 6 E and F


Diagnostic tool
PANR biomarker
- High LIF detection in PDA serum
Blood vessel
Schwann cells and neurons
- Neuronal plasticity
- Migration
- Differentiation
JAK/STAT3/AKT activation
LIF
LIFR
gp130
Schwann cell plasmic membrane
- LIF secretion
Macrophage
STROMA
Mast cell
Caf
Fibroblast

G

Figure 6 G

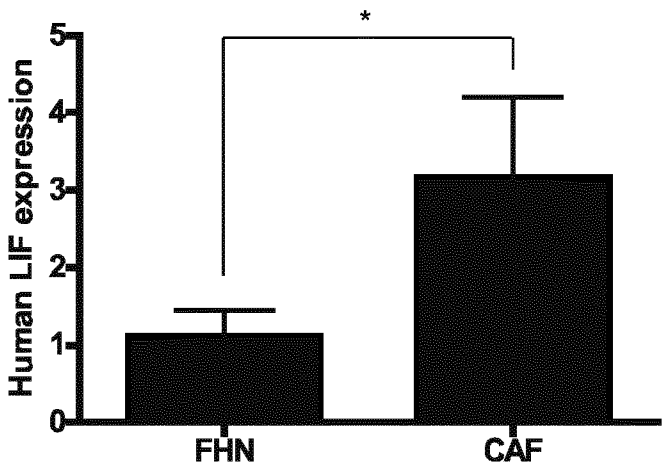


Figure 7

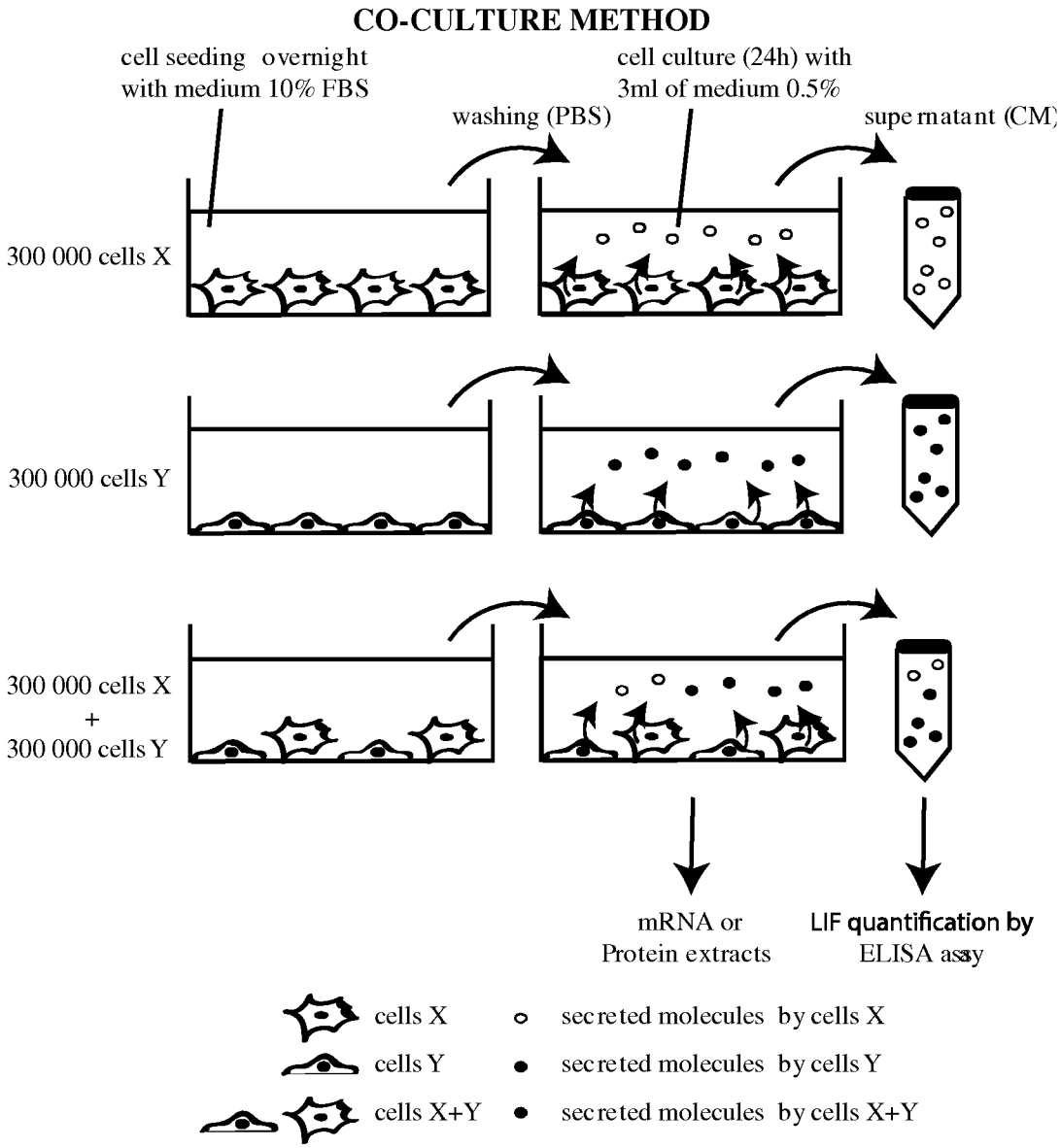


Figure 8A

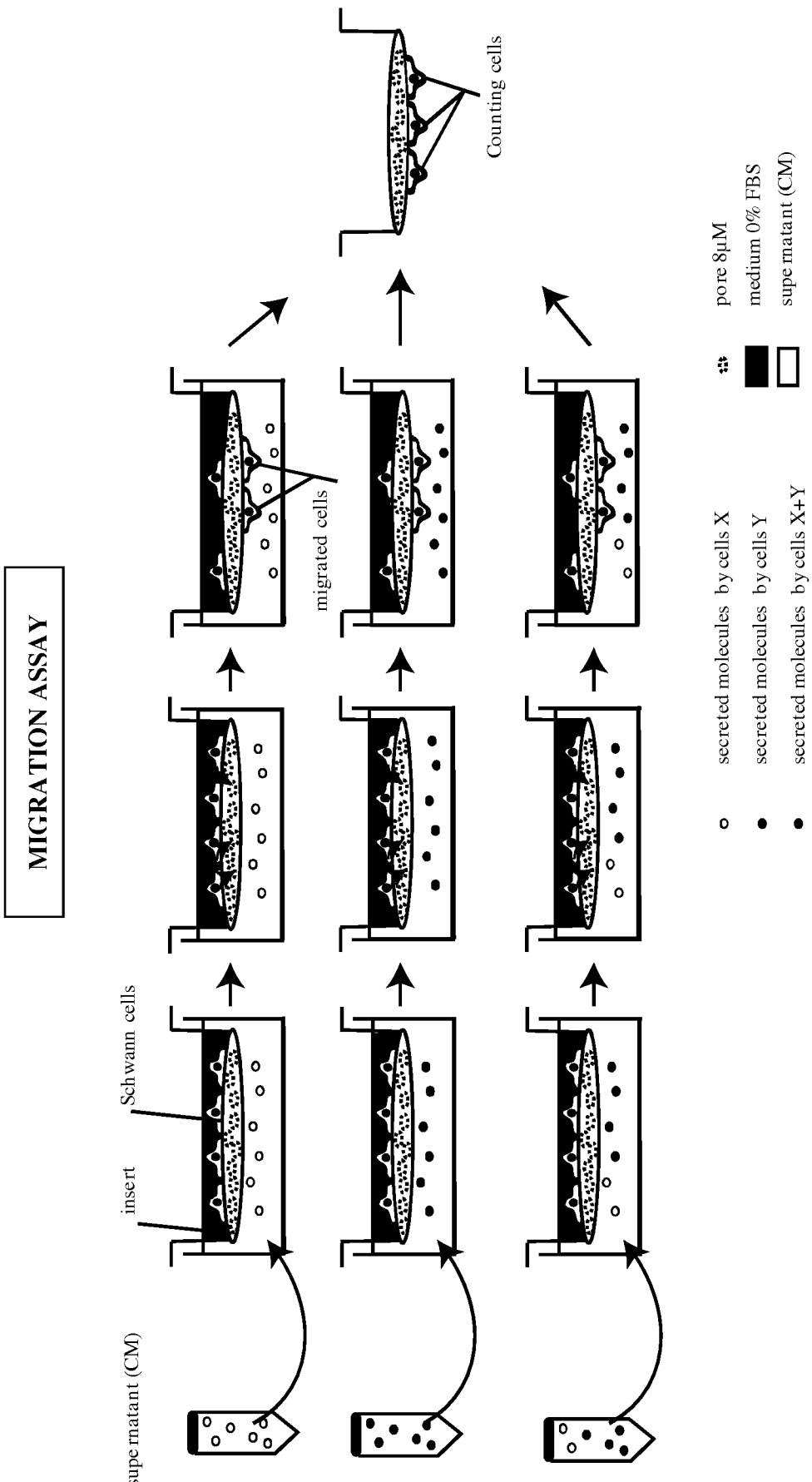

MIGRATION ASSAY
supernatant (CM)
insert
Schwann cells
migrated cells
Counting cells
secreted molecules by cells X
secreted molecules by cells Y
secreted molecules by cells X+Y
pore 8µM
medium 0% FBS
supernatant (CM)

Figure 8B

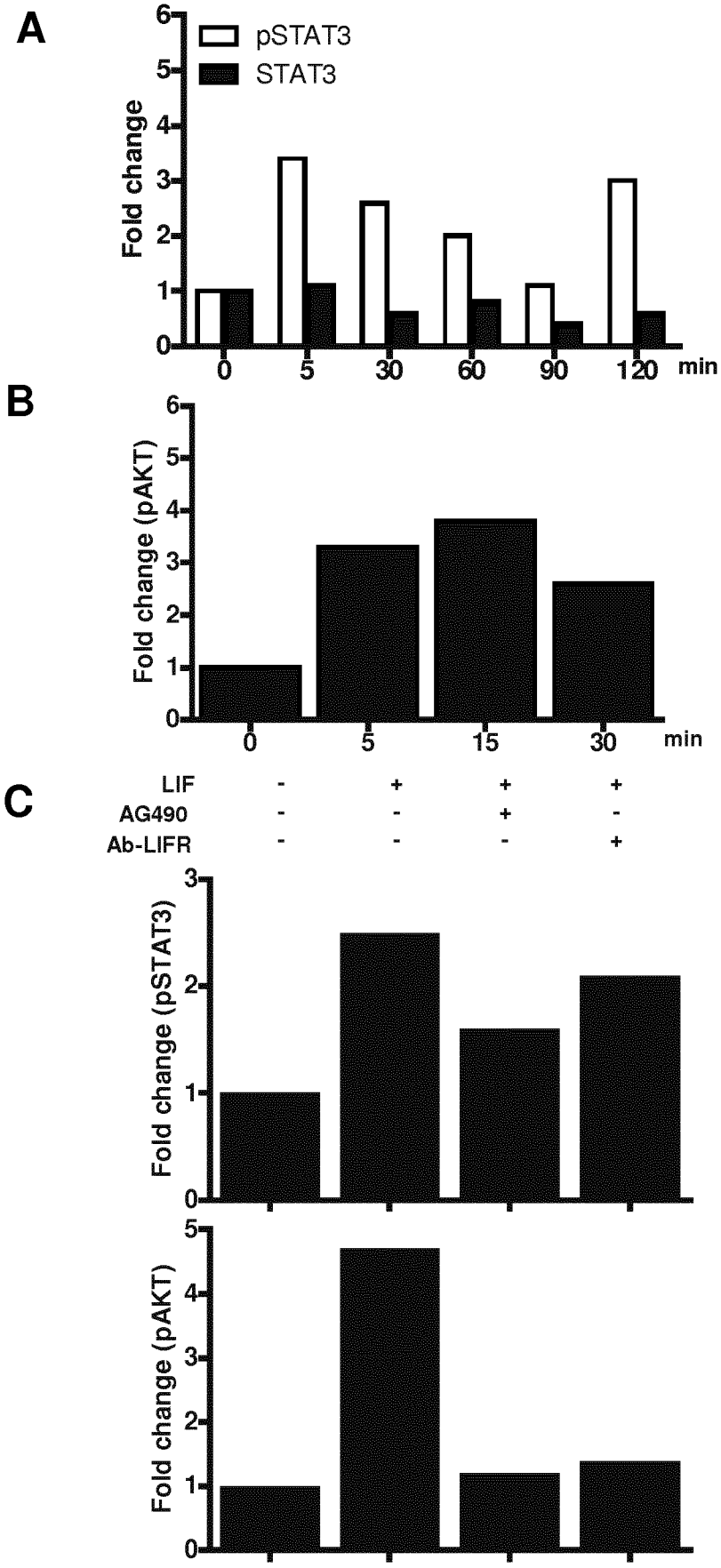


Figure 9 A, B and C

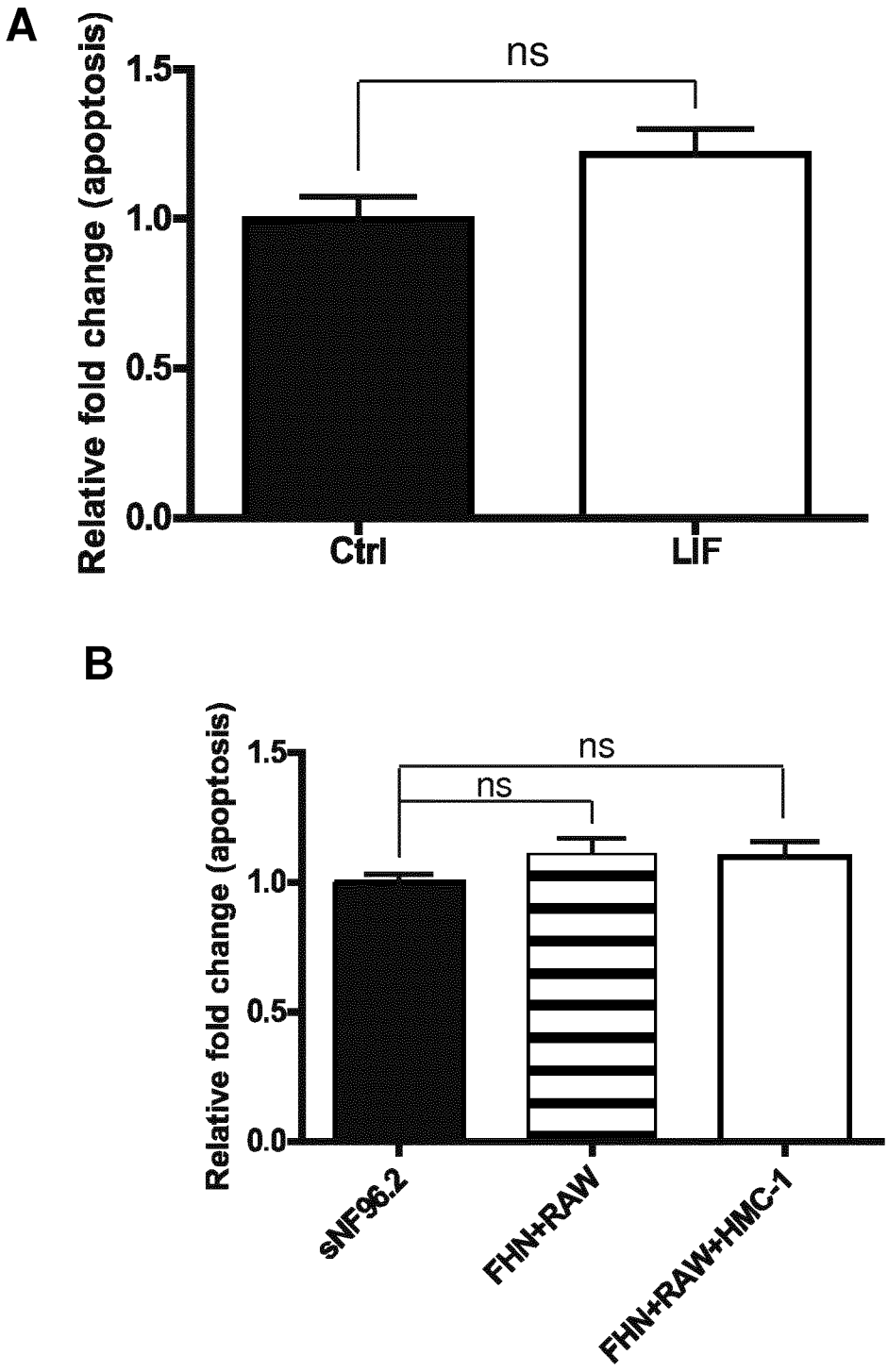


Figure 10 A and B

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/056706

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/4985 A61K31/277 A61K31/00 G01N33/48 G01N33/50
A61P1/18 A61P35/00
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K G01N A61P

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, SCISEARCH, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2010/065444 A1 (UNIV CENTRAL FLORIDA RES FOUND [US]; TURKSON JAMES [US]) 10 June 2010 (2010-06-10) in particular 'Summary of the Invention' Description of the figures Experimental Part 'Discussion' Claims ----- -/--	1-11

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 April 2018	04/05/2018

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Hornich-Paraf, E

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	US 2016/237158 A1 (GAGGIOLI CEDRIC [FR] ET AL) 18 August 2016 (2016-08-18) the whole document in particular paragraph [0005] - paragraph [0013] paragraph [0015] - paragraph [0019] paragraph [0025] - paragraph [0086] paragraph [0095] - paragraph [0101] paragraph [0108] - paragraph [0118] paragraph [0159] - paragraph [0171] paragraph [0289] - paragraph [0292] 'Discussion' Claims	1-11

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	S. XU ET AL: "Discovery of a Novel Orally Active Small-Molecule gp130 Inhibitor for the Treatment of Ovarian Cancer", MOLECULAR CANCER THERAPEUTICS, vol. 12, no. 6, 27 March 2013 (2013-03-27) , pages 937-949, XP055408156, US ISSN: 1535-7163, DOI: 10.1158/1535-7163.MCT-12-1082 abstract 'Materials and Methods' 'Results' 'Discussion'	1-11
A	IN AE SEO ET AL: "Janus Kinase 2 Inhibitor AG490 Inhibits the STAT3 Signaling Pathway by Suppressing Protein Translation of gp130", KOREAN JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY, vol. 13, no. 2, 1 January 2009 (2009-01-01), pages 131-138, XP055407737, KR ISSN: 1226-4512, DOI: 10.4196/kjpp.2009.13.2.131 in particular the abstract 'Discussion'	1-11

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	MCMURRAY ET AL: "A New Small-Molecule Stat3 Inhibitor", CHEMISTRY AND BIOLOGY, CURRENT BIOLOGY, LONDON, GB, vol. 13, no. 11, 1 November 2006 (2006-11-01), pages 1123-1124, XP027991106, ISSN: 1074-5521 [retrieved on 2006-11-01] the whole document	1-11
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X,P	CHRISTIAN BRESSY ET AL: "LIF Drives Neural Remodeling in Pancreatic Cancer and Offers a New Candidate Biomarker", CANCER RESEARCH, vol. 78, no. 4, 21 December 2017 (2017-12-21), pages 909-921, XP055469758, US ISSN: 0008-5472, DOI: 10.1158/0008-5472.CAN-15-2790 the whole document	1-11

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2018/056706

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		WO 2016179605 A1	10-11-2016