



(51) International Patent Classification:

A61K 38/22 (2006.01) A61P 35/00 (2006.01)
A61K 31/337 (2006.01)

(21) International Application Number:

PCT/EP2017/058499

(22) International Filing Date:

10 April 2017 (10.04.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

16305421.6 11 April 2016 (11.04.2016) EP

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

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: COMBINATION THERAPIES FOR THE TREATMENT OF PANCREATIC CANCER

(57) Abstract: The present invention relates to combination therapies for the treatment of pancreatic cancers. The inventors have compared and combined the effect of OxA and NAB-paclitaxel, on their anti-tumoral properties. Their results indicate that the addition of OxA and NAB-paclitaxel improves the effect of individual treatment and the sequential treatment consisting of first NAB-paclitaxel treatment followed by OxA treatment was more efficient than reverse treatment. OxA was close to NAB-paclitaxel treatment in term of response and suggest that combined treatment OxA/ NAB-paclitaxel represents a new promising pancreas cancer therapy. In particular, the present invention relates to an OX1R agonist in combination with taxane for use in the treatment of pancreatic cancer in a subject in need thereof.



COMBINATION THERAPIES FOR THE TREATMENT OF PANCREATIC CANCER

5 **FIELD OF THE INVENTION:**

The present invention relates to combination therapies for the treatment of pancreatic cancers.

BACKGROUND OF THE INVENTION:

10 Pancreatic cancer is an aggressive disease associated with an extremely poor prognosis. It is one of the most malignant cancers, characterized insidious onset, usually late diagnosis and low survival rate after diagnosis. For example, pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer death in the United States. In spite of recent therapeutic advances, long term survival in PDAC is often limited to patients
15 who have had surgery in early stage of the disease. The biological aggressiveness of PDAC is due, in part, to the tumor's resistance to chemotherapy. Presently, the standard of treatment remains systemic chemotherapy with gemcitabine, with palliative objectives and a disappointing marginal survival advantage. Very recently, the demonstration of a clinically and statistically meaningful survival advantage with the 5-fluorouracil, leucovorin, irinotecan
20 and oxaliplatin (FOLFIRINOX) regimen over single-agent gemcitabine (Conroy et al., N. Engl. J. Med., 364: 1817- 1825 (2011)). Another treatment recently developed consists in the introduction of nanoparticles of albumin-bound paclitaxel (nab-paclitaxel) to putatively target the desmoplastic stroma characteristic of pancreatic ductal adenocarcinoma (PDAC) (Garber, K., J. Natl. Cancer Inst., 102: 448-450 (2010)). These treatments have raised hope that
25 innovative combinations and improved delivery of classical cytotoxics may indeed substantially affect chemotherapy efficacy in advanced PDAC. Therefore, despite marginal advances in pancreatic cancer treatment, there remains a need for improved therapies and more creative approaches to devising and delivering effective pancreatic cancer therapies.

 The orexins (hypocretins) comprise two neuropeptides produced in the hypothalamus:
30 the orexin A (OX-A) (a 33 amino acid peptide) and the orexin B (OX-B) (a 28 amino acid peptide) (Sakurai T. et al., Cell, 1998, 92, 573-585). Orexins are found to stimulate food consumption in rats suggesting a physiological role for these peptides as mediators in the central feedback mechanism that regulates feeding behaviour. Orexins regulate states of sleep and wakefulness opening potentially novel therapeutic approaches for narcoleptic or

insomniac patients. Orexins have also been indicated as playing a role in arousal, reward, learning and memory. Two orexin receptors have been cloned and characterized in mammals. They belong to the super family of G-protein coupled receptors (7-transmembrane spanning receptor) (Sakurai T. et al., Cell, 1998, 92, 573-585): the orexin-1 receptor (OX1R or HCTR1) is selective for OX-A and the orexin-2 receptor (OX2R or HCTR2) is capable to bind OX-A as well as OX-B. A recent study shows that activation of OX1R by orexin can promote robust in vitro and in vivo apoptosis in colon cancer cells even when they are resistant to the most commonly used drug in colon cancer chemotherapy (Voisin T, El Firar A, Fasseu M, Rouyer-Fessard C, Descatoire V, Walker F, Paradis V, Bedossa P, Henin D, Lehy T, Laburthe M. Aberrant expression of OX1 receptors for orexins in colon cancers and liver metastases: an openable gate to apoptosis. Cancer Res. 2011 May 1;71(9):3341-51). Remarkably, all primary colorectal tumors regardless of their localization and Duke's stages expressed OX1R while adjacent normal colonocytes as well as control normal tissues were negative. Thus this study supports that OX1R is an Achilles's heel of colon cancers (even chemoresistance) and suggests that OX1R agonists might be novel candidates for colon cancer therapy. In the same way, WO 2015/071701 discloses use of OX1R agonists for the treatment of pancreatic cancers.

SUMMARY OF THE INVENTION:

The present invention relates to combination therapies for the treatment of pancreatic cancers. In particular, the invention is defined by the claims.

DETAILED DESCRIPTION OF THE INVENTION:

The inventors have previously demonstrated that OX1R was highly expressed in digestive cancers including cancer of colon (Voisin et al., Cancer research 2011, 71:3341-51), pancreas (Speisky et al., AACR annual meeting, 2014, San Diego, USA) and liver. In these cancers, orexin-A induces a mitochondrial apoptosis and a strong inhibition of tumor growth in nude mice xenografted with digestive cancer cell lines.

Here, the inventors have compared and combined the effect of OxA and NAB-paclitaxel, on their anti-tumoral properties. Their results indicate that the addition of OxA and NAB-paclitaxel improves the effect of individual treatment and the sequential treatment consisting of first NAB-paclitaxel treatment followed by OxA treatment was more efficient

than reverse treatment. OxA was close to NAB-paclitaxel treatment in term of response and suggest that combined treatment OxA/ NAB-paclitaxel represents a new promising pancreas cancer therapy.

5 A first aspect of the present invention relates to a method of treating pancreatic cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of an OX1R agonist in combination with a therapeutically effective amount of taxane.

10 A second aspect of the present invention relates to a method of treating a pancreatic cancer in a subject in need thereof comprising first administering to the subject a therapeutically effective amount of taxane, then administering to the subject a therapeutically effective amount of an OX1R agonist.

15 As used herein, the term "pancreatic cancer" or "pancreas cancer" relates to cancer which is derived from pancreatic cells. In particular, pancreatic cancer included pancreatic adenocarcinoma (e.g., pancreatic ductal adenocarcinoma) as well as other tumors of the exocrine pancreas (e.g., serous cystadenomas), acinar cell cancers, intraductal papillary mucinous neoplasms (IPMN) and pancreatic neuroendocrine tumors (such as insulinomas).

20 In one embodiment, the pancreatic cancer is selected from the group consisting of pancreatic adenocarcinoma such as pancreatic ductal adenocarcinoma and other tumors of the exocrine pancreas such as serous cystadenomas, acinar cell cancers, intraductal papillary mucinous neoplasms (IPMN) and pancreatic neuroendocrine tumors such as insulinomas.

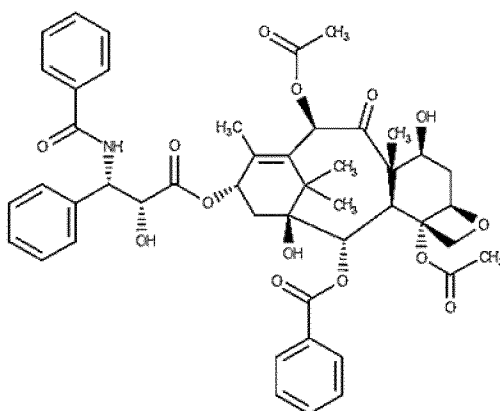
25 As used herein, the term "treatment" or "treat" refer to both prophylactic or preventive treatment as well as curative or disease modifying treatment, including treatment of patient at risk of contracting the disease or suspected to have contracted the disease as well as patients who are ill or have been diagnosed as suffering from a disease or medical condition, and includes suppression of clinical relapse. The treatment may be administered to a subject
30 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 patient 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 patient during treatment of an illness, e.g., to keep the patient 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 interval, 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., pain, disease manifestation, etc.]).

As used herein, the term "taxane" refers to any known taxane compound, or known taxane derivatives, or salts thereof. Taxanes are a class of diterpenes produced by the plants of the genus *Taxus* (yews). This term also includes those taxanes that have been artificially synthesized. Two classic taxane compounds widely used as chemotherapeutic agents are paclitaxel and docetaxel.

In a particular embodiment, the taxane is nab-paclitaxel.

As used herein, the term "paclitaxel" refers to the molecule of the following formula (I):



The crystal and molecular (I) structure of paclitaxel is well established (Crystal and molecular structure of paclitaxel (taxol). Donald Mastropaolo, Arthur Camerman, Yuogang Lu, Gary D. Brayer, and Norman Camerman. Proc. Natl. Acad. Sci. USA, Vol. 92, pp. 6920-6924, July 1995, Chemistry). Paclitaxel, also called “taxol”, is an anticancer drug that inhibits cell division by binding to and stabilizing microtubules polymers and protects it from disassembly. More precisely, paclitaxel targets tubulin. Paclitaxel, as antimetabolic agent, is used for the treatment of numerous cancers such as breast cancer, lung cancer or pancreatic cancer for instance.

As used herein, the terms “nanoparticle albumin-bound paclitaxel” and “nab-paclitaxel” refer to an albumin-bound form of paclitaxel. Nab-paclitaxel is a solvent-free formulation of paclitaxel bound to albumin.

Due to its hydrophobic properties, Paclitaxel is insoluble. Usually, solvents used are detergent-like substances, which are associated with toxicity, including peripheral neuropathy and hypersensitivity reactions. Paclitaxel coupled to albumin permits to convert an insoluble drug into an injectable form without using toxic solvents.

Indeed, albumin is natural carrier of hydrophobic molecules in body by binding to the glycoprotein receptor gp60 on endothelial cells, resulting in activation of caveolin-1 and the transcytosis of intact nanoparticles across the cell membrane. In addition to the active albumin-mediated transport of nab-paclitaxel into tumour cells, a degree of tumour-selective targeting is provided by SPARC, a protein which modulates the interaction of cells with the extracellular matrix. SPARC binds albumin with an affinity almost as great as that of gp60 and is over-expressed in many cancers (Nanoparticle albumin-bound (nabTM)-paclitaxel: improving efficacy and tolerability by targeted drug delivery in metastatic breast cancer. J. Cortes, C. Saura. Eur. J. Cancer, 8 (2010), pp. 1–10) (Purcell M, Neault JF, Tajmir-Riahi HA. Interaction of taxol with human serum albumin. Biochim Biophys Acta 2000;1478:61–68) (Paal K, Muller J, Hegedus L. High affinity binding of paclitaxel to human serum albumin. Eur J Biochem 2001;268:2187–91).

Nab-paclitaxel consists of particles which are surrounded by a hydrophilic exterior created by the negatively-charged amino acids in albumin proteins and which comprise a

hydrophobic core in interaction with Paclitaxel. Nab-paclitaxel is usually formulated in a colloidal suspension, due to the chemical properties of the albumin-paclitaxel particles. The diameter of nab-paclitaxel particles is comprised between 50 nanometres and 150 nanometres. Thus, the use of albumin particles as carriers in nab-paclitaxel's formulation improves tolerability by eliminating the need for chemical solvents, but also drug bioavailability (Desai N, Trieu V, Yao Z, Louie L, Ci S, Yang A. et al. Increased antitumor activity, intratumor paclitaxel concentrations, and endothelial cell transport of cremophor-free, albumin-bound paclitaxel, ABI-007, compared with cremophor-based paclitaxel. Clin Cancer Res. 2006; 12:1317-24). Indeed, nab-paclitaxel particles dissociate within 30 to 45 seconds of injection into their constituent albumin molecules which circulate rapidly around the body with their bound paclitaxel attached. This dissociation into individual drug-bound albumin molecules is concentration dependent.

As used herein, the term "OX1R" has its general meaning in the art and refers to the 7-transmembrane spanning receptor OX1R for orexins. According to the invention, OX1R promotes apoptosis in the human pancreatic cancer cell line through a mechanism which is not related to Gq-mediated phospholipase C activation and cellular calcium transients. Orexins induce indeed tyrosine phosphorylation of 2 tyrosine-based motifs in OX1R, ITIM and ITSM, resulting in the recruitment of the phosphotyrosine phosphatase SHP-2, the activation of which is responsible for mitochondrial apoptosis (Voisin T, El Firar A, Rouyer-Fessard C, Gratio V, Laburthe M. A hallmark of immunoreceptor, the tyrosine-based inhibitory motif ITIM, is present in the G protein-coupled receptor OX1R for orexins and drives apoptosis: a novel mechanism. FASEB J. 2008 Jun;22(6):1993-2002.; El Firar A, Voisin T, Rouyer-Fessard C, Ostuni MA, Couvineau A, Laburthe M. Discovery of a functional immunoreceptor tyrosine-based switch motif in a 7-transmembrane-spanning receptor: role in the orexin receptor OX1R-driven apoptosis. FASEB J. 2009 Dec;23(12):4069-80. doi: 10.1096/fj.09-131367. Epub 2009 Aug 6.). An exemplary amino acid sequence of OX1R is shown as SEQ ID NO:1.

orexin receptor-1 OX1R (SEQ ID NO:1)

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1 mepsatpgaq mgvppgsrep spvppdyede flrylwrdyl ypkqyewvli aayvavfvva
61 lvgntlvcla vwrnhhmrtv tnyfivnlsl advltaiel pasllvdite swlfghalck
121 vipylqavsv svavltlsfi aldrwyaich pllfkstarr argsilgiwa vsclaimvpqa
181 avmecssvlp elanrtrlfs vderwaddl ypkihscff ivtylaplgl mamayfqifr

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241 klwgrqipgt tsalvrnwkr psdqlgdleq glsgepqprg raflaevkqm rarrktakml
301 mvvllvfalc ylpisvlnvl krvgmfrqa sdreavyacf tfshwlvyan saanpiiyntf
361 lsgkfreqfk aafscclpgl gpcgslkaps prssashksl slqsrcsisk ischvvltsv
421 ttvlp

5

Accordingly, as used herein, the term "OX1R agonist" refers to any compound natural or not that is able to bind to OX1R and promotes OX1R activity which consists of activation of signal transduction pathways involving recruitment of SHP-2 and the induction of apoptosis of the cell, independently of transient calcium release.

10

In some embodiments, the OX1R agonist is a small organic molecule. The term "small organic molecule" refers to a molecule of a size comparable to those organic molecules generally used in pharmaceuticals. The term excludes biological macromolecules (e. g., proteins, nucleic acids, etc.). Preferred small organic molecules range in size up to about 5000 Da, more In particular up to 2000 Da, and most In particular up to about 1000 Da.

15

In some embodiment, the OX1R agonist is an antibody or a portion thereof.

20

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.

25

In one embodiment, the OX1R agonist is selected from the group consisting of chimeric antibodies, humanized antibodies or full human monoclonal antibodies.

30

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.

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 OX1R. 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 recombinant OX1R may be provided by expression with recombinant cell lines. In particular, OX1R may be provided in the form of human cells expressing OX1R at their surface. 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 hybridoma. 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
5 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
10 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,
15 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
20 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
25 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,
30 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.

5 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
10 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 be 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
15 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
20 use the donor amino acid residue at the framework positions at which the amino acid is predicted to have a side chain atom within 3Å 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
25 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
30 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 IgG1, 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.

5 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
10 (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
15 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
20 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
25 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
30 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 IgG1, IgG2, IgG3 and IgG4.

5

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
10 the invention, sdAb can particularly be llama sdAb.

In one embodiment, the OX1R agonist is a polypeptide. In one embodiment, the OX1R agonist is Orexin-A or Orexin-B.

15

As used herein the term "orexin-A" has its general meaning in the art and refers to the amino acid sequence as shown by SEQ ID NO:2.

Orexin-A (SEQ ID NO:2): peplpdccrqk tcsclryell hgagnhaagi ltlx (where "pe" stands for "pyroglutamic acid" and "x" stands for any amino acid).

20

As used herein the term "orexin-B" has its general meaning in the art and refers to the amino acid sequence as shown by SEQ ID NO:3.

Orexin-B (SEQ ID NO:3): rsgppglqgr lqrlqasgn haagiltm

25

Agonistic activity of the polypeptide is assessed by any assay well known in the art. After 24 hr culture, cells are treated with or without the polypeptide to be tested. After 48 hr of treatment, adherent cells were harvested by TrypLE (Life Technologies, Saint Aubin, France). Apoptosis is then determined using the Guava PCA system and the Guava nexin kit
30 as previously described (Voisin et al., 2008). Results are expressed as the percentage of apoptotic annexin V-phycoerythrin (PE)-positive cells. Typically, the apoptosis induction (EC50) of the polypeptide of the present invention ranges from 10 nM to 110 nM. More particularly, the apoptosis induction (EC50) of the polypeptide of the present invention ranges

from 10 nM to 50 nM. More particularly, the apoptosis induction (EC50) of the polypeptide of the present invention ranges from 15 nM to 30 nM.

5 In some embodiments, the polypeptide of the present invention is the functional equivalent of Orexin-A.

In some embodiments, the polypeptide of the present invention is the functional equivalent of Orexin-B.

10 As used herein, a "functional equivalent of orexin" is a polypeptide which is capable of binding to OX1R, thereby promoting an OX1R activity according to the invention. The term "functional equivalent" includes fragments, mutants, and muteins of Orexin-A and Orexin-B. The term "functionally equivalent" thus includes any equivalent of orexins (i.e. Orexin-A or Orexin-B) obtained by altering the amino acid sequence, for example by one or
15 more amino acid deletions, substitutions or additions such that the protein analogue retains the ability to bind to OX1R and promote an OX1R activity according to the invention (e.g. apoptosis of the cancer cell). Amino acid substitutions may be made, for example, by point mutation of the DNA encoding the amino acid sequence.

20 In some embodiments, the functional equivalent is at least 80% homologous to the corresponding protein. In a preferred embodiment, the functional equivalent is at least 90% homologous as assessed by any conventional analysis algorithm such as for example, the Pileup sequence analysis software (Program Manual for the Wisconsin Package, 1996). The term "a functionally equivalent fragment" as used herein also may mean any fragment or
25 assembly of fragments of Orexin that binds to OX1R and promote the OX1R activity according to the invention. Accordingly the present invention provides a polypeptide which comprises consecutive amino acids having a sequence which corresponds to the sequence of at least a portion of Orexin-A or Orexin-B, which portion binds to OX1R and promotes the OX1R activity according to the invention.

30

Functionally equivalent fragments may belong to the same protein family as the human Orexins identified herein. By "protein family" is meant a group of proteins that share a common function and exhibit common sequence homology. Homologous proteins may be derived from non-human species. In particular, the homology between functionally equivalent

protein sequences is at least 25% across the whole of amino acid sequence of the complete protein. More In particular, the homology is at least 50%, even more In particular 75% across the whole of amino acid sequence of the protein or protein fragment. More In particular, homology is greater than 80% across the whole of the sequence. More In particular, homology is greater than 90% across the whole of the sequence. More In particular, homology is greater than 95% across the whole of the sequence.

In one embodiment, the OX1R agonist of the present invention is a polypeptide having at least 80% of identity with SEQ ID NO:2 or SEQ ID NO:3.

In a particular embodiment, the polypeptide of the present invention comprises the amino acid sequence ranging from the amino acid residue at position 6 to the amino acid residue at position 28 in SEQ ID NO:3 wherein at least one amino acid residue position 6, 7, 8, 9, 10, 12, 13, 14, 19, 21 or 23 is substituted and the amino acid residues at position 11; 15; 16; 17; 18; 20; 22; 24; 25; 26; 27; and 28 are not deleted or substituted.

As used herein, the term "substitution" means that a specific amino acid residue at a specific position is removed and another amino acid residue is inserted into the same position.

In some embodiments, the amino acid residue at position 6, 7, 8, 9, 10, 12, 13, 14, 19, 21, or 23 is substituted by an alanine.

In some embodiments, the substitution is a conservative substitution. In the context of the present invention, a "conservative substitution" is defined by substitutions within the classes of amino acids reflected as follows:

Aliphatic residues I, L, V, and M

Cycloalkenyl-associated residues F, H, W, and Y

Hydrophobic residues A, C, F, G, H, I, L, M, R, T, V, W, and Y

Negatively charged residues D and E

Polar residues C, D, E, H, K, N, Q, R, S, and T

Positively charged residues H, K, and R

Small residues A, C, D, G, N, P, S, T, and V

Very small residues A, G, and S

Residues involved in turn A, C, D, E, G, H, K, N, Q, R, S, P, and formation T

Flexible residues Q, T, K, S, G, P, D, E, and R

More conservative substitutions groupings include: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine. Conservation in terms of hydropathic/hydrophilic properties and residue weight/size also is substantially retained in the polypeptide of the present invention as compared to the native sequence of Orxin-B. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art. It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8) ; phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophane (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5). The retention of similar residues may also or alternatively be measured by a similarity score, as determined by use of a BLAST program (e.g., BLAST 2.2.8 available through the NCBI using standard settings BLOSUM62, Open Gap= 11 and Extended Gap= 1).

In some embodiments, the polypeptide of the present invention comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 substitutions in the amino acid sequence ranging from the amino acid residue at position 6 to the amino acid residue at position 28 in SEQ ID NO:3.

In some embodiments, the methionine residue at position 28 is amidated. As used herein, the term "amidation," has its general meaning in the art and refers to the process consisting of producing an amide moiety.

In some embodiments, the polypeptide of the present invention is fused to a heterologous polypeptide to form a fusion protein. As used herein, a "fusion protein" comprises all or part (typically biologically active) of a polypeptide of the present invention operably linked to a heterologous polypeptide (i.e., a polypeptide other than the same polypeptide). Within the fusion protein, the term "operably linked" is intended to indicate that the polypeptide of the present invention and the heterologous polypeptide are fused in-frame to each other. The heterologous polypeptide can be fused to the N-terminus or C-terminus of

the polypeptide of the present invention. In some embodiment, the heterologous polypeptide is fused to the C-terminal end of the polypeptide of the present invention.

In some embodiments, the polypeptide of the present invention and the heterologous polypeptide are fused to each other directly (i.e. without use of a linker) or via a linker. The linker is typically a linker peptide and will, according to the invention, be selected so as to allow binding of the polypeptide to the heterologous polypeptide. Suitable linkers will be clear to the skilled person based on the disclosure herein, optionally after some limited degree of routine experimentation. Suitable linkers are described herein and may - for example and without limitation - comprise an amino acid sequence, which amino acid sequence preferably has a length of 2 or more amino acids. Typically, the linker has 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 amino acids. However, the upper limit is not critical but is chosen for reasons of convenience regarding e.g. biopharmaceutical production of such fusion proteins. The linker sequence may be a naturally occurring sequence or a non-naturally occurring sequence. If used for therapeutical purposes, the linker is preferably non-immunogenic in the subject to which the fusion protein of the present invention is administered. One useful group of linker sequences are linkers derived from the hinge region of heavy chain antibodies as described in WO 96/34103 and WO 94/04678. Other examples are poly-alanine linker sequences such as Ala-Ala-Ala. Further preferred examples of linker sequences are Gly/Ser linkers of different length including (gly4ser)₃, (gly4ser)₄, (gly4ser), (gly3ser), gly₃, and (gly3ser)₂.

In one embodiment, the OX1R agonist is an immunoadhesin.

In some embodiments, the polypeptide of the present invention is fused to an immunoglobulin domain. For example the fusion protein of the present invention may comprise a polypeptide of the present invention that is fused to an Fc portion (such as a human Fc) to form an immunoadhesin. As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding specificity of a heterologous protein (an "adhesin" which is able to bind to OX1R) with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins comprise a fusion of the polypeptide of the present invention and an immunoglobulin constant domain sequence. The immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3, or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM. The immunoglobulin sequence typically, but not necessarily, is an

immunoglobulin constant domain (Fc region). Immunoadhesins can possess many of the valuable chemical and biological properties of human antibodies. Since immunoadhesins can be constructed from a human protein sequence with a desired specificity linked to an appropriate human immunoglobulin hinge and constant domain (Fc) sequence, the binding specificity of interest can be achieved using entirely human components. Such immunoadhesins are minimally immunogenic to the patient, and are safe for chronic or repeated use. In some embodiments, the Fc region is a native sequence Fc region. In some embodiments, the Fc region is a variant Fc region. In still another embodiment, the Fc region is a functional Fc region. As used herein, the term "Fc region" is used to define a C-terminal region of an immunoglobulin heavy chain, including native sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The adhesion portion and the immunoglobulin sequence portion of the immunoadhesin may be linked by a minimal linker. The immunoglobulin sequence typically, but not necessarily, is an immunoglobulin constant domain. The immunoglobulin moiety in the chimeras of the present invention may be obtained from IgG1, IgG2, IgG3 or IgG4 subtypes, IgA, IgE, IgD or IgM, but typically IgG1 or IgG3.

The polypeptides of the present invention can exhibit post-translational modifications, including, but not limited to glycosylations, (e.g., N-linked or O-linked glycosylations), myristylations, palmitylations, acetylations and phosphorylations (e.g., serine/threonine or tyrosine). In some embodiments, it is contemplated that polypeptides used in the therapeutic methods of the present invention may be modified in order to improve their therapeutic efficacy. Such modification of therapeutic compounds may be used to decrease toxicity, increase circulatory time, or modify biodistribution. For example, the toxicity of potentially important therapeutic compounds can be decreased significantly by combination with a variety of drug carrier vehicles that modify biodistribution. In example adding dipeptides can improve the penetration of a circulating agent in the eye through the blood retinal barrier by using endogenous transporters. A strategy for improving drug viability is the utilization of water-soluble polymers. Various water-soluble polymers have been shown to modify biodistribution, improve the mode of cellular uptake, change the permeability through physiological barriers; and modify the rate of clearance from the body. To achieve either a targeting or sustained-release effect, water-soluble polymers have been synthesized that

contain drug moieties as terminal groups, as part of the backbone, or as pendent groups on the polymer chain. Polyethylene glycol (PEG) has been widely used as a drug carrier, given its high degree of biocompatibility and ease of modification. Attachment to various drugs, proteins, and liposomes has been shown to improve residence time and decrease toxicity.

- 5 PEG can be coupled to active agents through the hydroxyl groups at the ends of the chain and via other chemical methods; however, PEG itself is limited to at most two active agents per molecule. In a different approach, copolymers of PEG and amino acids were explored as novel biomaterials which would retain the biocompatibility properties of PEG, but which would have the added advantage of numerous attachment points per molecule (providing
- 10 greater drug loading), and which could be synthetically designed to suit a variety of applications. Those of skill in the art are aware of PEGylation techniques for the effective modification of drugs. For example, drug delivery polymers that consist of alternating polymers of PEG and tri-functional monomers such as lysine have been used by VectraMed (Plainsboro, N.J.). The PEG chains (typically 2000 daltons or less) are linked to the α - and ϵ -
- 15 amino groups of lysine through stable urethane linkages. Such copolymers retain the desirable properties of PEG, while providing reactive pendent groups (the carboxylic acid groups of lysine) at strictly controlled and predetermined intervals along the polymer chain. The reactive pendent groups can be used for derivatization, cross-linking, or conjugation with other molecules. These polymers are useful in producing stable, long-circulating pro-drugs by
- 20 varying the molecular weight of the polymer, the molecular weight of the PEG segments, and the cleavable linkage between the drug and the polymer. The molecular weight of the PEG segments affects the spacing of the drug/linking group complex and the amount of drug per molecular weight of conjugate (smaller PEG segments provides greater drug loading). In general, increasing the overall molecular weight of the block co-polymer conjugate will
- 25 increase the circulatory half-life of the conjugate. Nevertheless, the conjugate must either be readily degradable or have a molecular weight below the threshold-limiting glomular filtration (e.g., less than 60 kDa). In addition, to the polymer backbone being important in maintaining circulatory half-life, and biodistribution, linkers may be used to maintain the therapeutic agent in a pro-drug form until released from the backbone polymer by a specific
- 30 trigger, typically enzyme activity in the targeted tissue. For example, this type of tissue activated drug delivery is particularly useful where delivery to a specific site of biodistribution is required and the therapeutic agent is released at or near the site of pathology. Linking group libraries for use in activated drug delivery are known to those of skill in the art and may be based on enzyme kinetics, prevalence of active enzyme, and

cleavage specificity of the selected disease-specific enzymes. Such linkers may be used in modifying the protein or fragment of the protein described herein for therapeutic delivery.

The polypeptides of the present invention may be produced by any suitable means, as will be apparent to those of skill in the art. In order to produce sufficient amounts of polypeptides or functional equivalents thereof for use in accordance with the present invention, expression may conveniently be achieved by culturing under appropriate conditions recombinant host cells containing the polypeptide of the present invention. In particular, the polypeptide is produced by recombinant means, by expression from an encoding nucleic acid molecule. Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. When expressed in recombinant form, the polypeptide is in particular generated by expression from an encoding nucleic acid in a host cell. Any host cell may be used, depending upon the individual requirements of a particular system. Suitable host cells include bacteria mammalian cells, plant cells, yeast and baculovirus systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary cells, HeLa cells, baby hamster kidney cells and many others. Bacteria are also preferred hosts for the production of recombinant protein, due to the ease with which bacteria may be manipulated and grown. A common, preferred bacterial host is E coli.

In specific embodiments, it is contemplated that polypeptides used in the therapeutic methods of the present invention may be modified in order to improve their therapeutic efficacy. Such modification of therapeutic compounds may be used to decrease toxicity, increase circulatory time, or modify biodistribution. For example, the toxicity of potentially important therapeutic compounds can be decreased significantly by combination with a variety of drug carrier vehicles that modify biodistribution. In example adding dipeptides can improve the penetration of a circulating agent in the eye through the blood retinal barrier by using endogenous transporters.

In one embodiment, the OX1R agonist is an aptamer. Aptamers are a class of molecule that represents an alternative to antibodies in term of molecular recognition. Aptamers are oligonucleotide or oligopeptide 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. 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. Peptide aptamers consists of a conformationally constrained antibody variable region displayed by a platform protein, such as E. coli Thioredoxin A that are
5 selected from combinatorial libraries by two hybrid methods.

An aspect of the present invention relates to a method for the treatment of pancreatic cancer in a subject in need thereof comprising administering the subject with a therapeutically effective amount of an OX1R agonist in combination with a therapeutically effective amount
10 of taxane.

By a "therapeutically effective amount" is meant a sufficient amount of OX1R agonist or taxane to treat pancreatic cancer at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood that the total daily usage of the compounds and compositions
15 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 compound employed; the specific composition employed, the age, body weight, general health, sex and diet of the subject; the time of administration,
20 route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific polypeptide 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 compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired
25 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. In particular, 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 active ingredient 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 active ingredient, in particular from 1 mg
30 to about 100 mg of the active ingredient. 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.

As used herein the term "in combination" as used herein means a process whereby the combination of the OX1R agonist and taxane, is administered to the same patient. OXR1 agonist and taxane may be administered simultaneously, at essentially the same time, or sequentially. If administration takes place sequentially, taxane is administered before OXR1 agonist.

In another embodiment, the OX1R agonist and taxane are administered simultaneously, at essentially the same time, or sequentially.

In some embodiments, the OX1R agonist and taxane are administered sequentially.

In some embodiments, the method of the present invention comprises first administering the subject with a therapeutically effective amount of taxane, then administering the subject with a therapeutically effective amount of an OX1R agonist.

Typically, the duration of the treatment with taxane is comprised between 10 and 50 days. In one embodiment, the duration of the treatment with taxane is more preferably comprised between 25 and 35 days.

In particular, the duration of the treatment with taxane may be equal to 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 days.

Typically the duration of the treatment with OX1R agonist is comprised between 10 and 50 days. In one embodiment, the duration of the treatment with OX1R agonist is more preferably comprised between 25 and 35 days.

In particular, the duration of the treatment with OX1R agonist may be equal to 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 days.

In some embodiments, taxane and the OXR1 agonist may be administered one or more times and the number of administrations of each component of the combination may be the same or different. In a particular embodiment, 2 intraperitoneal injections of OX1R agonist and/or 2 intraperitoneal injections of taxane per week were performed.

In some embodiments, the time lapse between taxane treatment and OXR1 agonist treatment is equal to zero (e.g. administration at the same time) or may be comprised between 3 and 4 days.

5 Typically, the OXR1 agonist and taxane of the invention may be combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form therapeutic compositions.

10 "Pharmaceutically" or "pharmaceutically acceptable" refers 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.

15 The compositions according to the invention are formulated for parenteral, transdermal, oral, rectal, subcutaneous, sublingual, intrapulmonary, topical or intranasal administration.

Suitable unit administration forms comprise oral-route forms such as tablets, gel capsules, powders, granules and oral suspensions or solutions, sublingual and buccal
20 administration forms, aerosols, implants, subcutaneous, transdermal, topical, intraperitoneal, intramuscular, intravenous, subdermal, transdermal, intrathecal and intranasal administration forms and rectal administration forms.

In particular, the pharmaceutical compositions are formulated for parenteral
25 administration. 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, potassium, 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
30 water or physiological saline, permit the constitution of injectable solutions.

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: Effect of NAB-paclitaxel (pacli) and orexin-A (OxA) on the growth (panel A) and the apoptosis (panel B) of AsPC-1 cells.**

- 5 A, Cells were treated for 48h with 0.1 μ M of each compound and then cells were counted. Results were expressed as the percentage of untreated cell number (Control).
- B, AsPC-1 cells were challenged with 0.1 μ M of each compound for 48h. Apoptosis was measured by determination of annexin V-PE binding, and results are expressed as the percentage of apoptotic cells.

10

Figure 2: Effect of NAB-paclitaxel (pacli), orexin-A (OxA) and pacli/OxA sequential treatment on the growth of AsPC-1 cells.

Cells were treated for 96h with 0.1 μ M of each compound or sequentially for 48h with 0.1 μ M of each compound and then cells were counted.

- 15 Results were expressed as the percentage of untreated cell number (Control).

Figure 3: Effect of inoculation of OxA, NAB-paclitaxel (pacli) and mixed OxA+paclitaxel on the Growth of tumors developed by xenografted human AsPC-1 cells in nude mice.

- 20 Pancreatic adenocarcinoma derived cells, AsPC-1, were inoculated in the flank of nude mice at day 0. Mice were injected at day 1 (2 injections/week) intraperitoneally with 100 μ l of OxA solution (1.12 μ moles of OxA/Kg (white circles)) or with 100 μ l of pacli solution (1.12 μ moles of OxA/Kg (black triangles)) or with 100 μ l of mixed two compounds (white triangles) or with 100 μ l of PBS (black circles) for control.

25

Figure 4: Effect of inoculation of OxA and sequential treatment (OxA/NAB-paclitaxel or NAB-paclitaxel/OxA) on the growth of tumors developed by xenografted human AsPC-1 cells in nude mice.

- 30 Pancreatic adenocarcinoma derived cells, AsPC-1, were inoculated in the flank of nude mice at day 0. Mice were injected at day 1 (2 injections/week) intraperitoneally with 100 μ l of OxA solution (1.12 μ moles of OxA/Kg (black triangles)) or with 100 μ l of OxA for 30 days followed by 30 days with NAB-paclitaxel (1.12 μ moles/Kg (white squares)) or with 100 μ l of NAB-paclitaxel followed by 30 days of OxA (black squares) or 100 μ l of PBS (black circles) for control.

EXAMPLE:

In the present work, the inventors have compared and combined the effect of Orexin-A and NAB-paclitaxel which represents the “gold standard” reference in the chemotherapeutic treatment of pancreas cancer, on their anti-tumoral properties.

5

Material & Methods**Cells growth determination and apoptosis assay**

Pancreas adenocarcinoma AsPC-1 cells were seeded, grown and maintained at 37°C in a humidified 5% CO₂/air incubator. After 24 hr culture, cells were treated with or without
10 Orexin-A peptide or NAB-paclitaxel to be tested at the concentration indicated in the figure legends. After 48 hr of treatment, adherent cells were harvested by TripleE (Life Technologies, Saint Aubin, France) and manually counted. Apoptosis was determined using the Guava PCA system and the Guava nexin kit.

Tumorigenicity assay in nude mice

15 Exponentially growing AsPC-1 cells were harvested, washed with PBS and then resuspended in gelatin (2% solution type B from bovine skin, Sigma). Nude mice were anesthetized by intraperitoneal injection of a mixture containing 25µL of Rompun 2% (Xylazine, Bayer) and 200µL of Imalgene 500 (Ketamine 50 mg/mL, Merial) in 400µL of PBS. Cells (106/100 µL) were then inoculated subcutaneously into the flank of mice. All nude
20 mice developed tumors at the site of inoculation between day 3 and 10. Tumor development was followed by caliper measurements in 2 dimensions (L and W), and the volume (V) of the tumor was calculated with the formula for a prolate ellipsoid ($V = L \times W^2 \times \pi/6$) as reported (21)(22). For treatment with orexin-A (GL Biochemicals), the compounds were dissolved in PBS, and 1.12 µmol/kg of body weight were administered by intraperitoneal injections.
25 Control mice received PBS. No adverse effect of orexin-A or NAB-paclitaxel could be observed during treatment. At the end of the in vivo experiments, mice were necropsied. The xenografted tumors were then resected, weighed (MARK electronic balance, Bel engineering).

30

Results

The incubation of AsPC-1 pancreatic cancer cell line which expressed OX1R, with 0.1µM OxA or 0.1 µM NAB-paclitaxel reveals a cell growth inhibition of 36% and 51%, respectively. The addition of 0.1 µM OxA and 0.1µM NAB-paclitaxel on AsPC-1 cells reveals a significantly cell growth inhibition of 70% suggesting that the double treatment was

more efficient than individual treatment. Moreover, the addition of 0.1 μ M OxA and 0.1 μ M NAB-paclitaxel induces 25% of cell apoptosis determined by annexin-V labeling, as compared to single treatment with 0.1 μ M OxA (18%) or 0.1 μ M NAB-paclitaxel (12%). Additionally, we explore the sequential treatment by OxA and NAB-paclitaxel on cell growth of AsPC-1 cells. Our results evidenced that 48h treatment by OxA followed by 48h treatment of NAB-paclitaxel induced an inhibition of 35% of cell growth. In contrast, the reverse treatment (48h NAB-paclitaxel followed by 48h OxA) induces an inhibition of cell growth of 60%. OxA intraperitoneal injection (2 injections/week of 1.12 μ moles/kg OxA and/or NAB-paclitaxel) in nude mice xenografted with AsPC-1 cells, shows that OxA and NAB-paclitaxel induces an inhibition of tumoral volume of 60% and 62%, respectively. Moreover, injection of OxA plus NAB-paclitaxel induces an inhibition of tumoral volume of 70%. Sequential treatments of xenografted tumors in mice with OxA and NAB-paclitaxel was investigated and revealed 72% tumor growth inhibition when mice were treated 30 days with OxA followed by 30 days with NAB-paclitaxel and 83 % tumor growth inhibition when they were treated 30 days with NAB-paclitaxel followed by 30 days with OxA. These results indicate that: 1) the addition of OxA and NAB-paclitaxel improves the effect of individual treatment; 2) the sequential treatment consisting of first NAB-paclitaxel treatment followed by OxA treatment was more efficient than reverse treatment. In conclusion, OxA was close to NAB-paclitaxel treatment in term of response and suggest that combined treatment OxA/ NAB-paclitaxel represents a new promising pancreas cancer therapy.

REFERENCES:

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.

CLAIMS:

1. A method for the treatment of pancreatic cancer in a subject in need thereof comprising administering the subject with a therapeutically effective amount of an OX1R agonist in
5 combination with a therapeutically effective amount of taxane.
2. The method according to claim 1, wherein the therapeutically effective amount of an OX1R agonist and the therapeutically effective amount of taxane are administered simultaneously, at essentially the same time, or sequentially.
3. The method according to claim 2, wherein the therapeutically effective amount of an OX1R
10 agonist and the therapeutically effective amount of taxane are administered sequentially.
4. The method according to claim 3 wherein comprising first administering the subject with a therapeutically effective amount of taxane, then administering the subject with a therapeutically effective amount of an OX1R agonist.
5. The method according to one of the preceding claims, wherein the duration of the treatment
15 with taxane is comprised between 10 and 50 days.
6. The method according to claim 5, wherein the duration of the treatment with taxane is more preferably comprised between 25 and 35 days.
7. The method according to one of the preceding claims, wherein the duration of the treatment with OX1R agonist is comprised between 10 and 50 days.
- 20 8. The method according to claim 7 wherein the duration of the treatment with OX1R agonist is more preferably comprised between 25 and 35 days.
9. The method according to one of the preceding claims, wherein the taxane is nab-paclitaxel.
10. The method according to one of the preceding claims, wherein the OX1R agonist is a small organic molecule.
- 25 11. The method according to one of the preceding claims, wherein the OX1R agonist is an antibody.

12. The method according to one of the preceding claims, wherein the OX1R agonist is selected from the group consisting of chimeric antibodies, humanized antibodies or full human monoclonal antibodies.

5 13. The method according to one of the preceding claims, wherein the OX1R agonist is a polypeptide.

14. The method according to claim 13, wherein the OX1R agonist is Orexin-A or Orexin-B.

15. The method according to one of the preceding claims, wherein the OX1R agonist is a functional equivalent of Orexin-A or Orexin-B.

10 16. The method according to one of the preceding claims, wherein the OX1R agonist is a polypeptide having at least 80% of identity with SEQ ID NO:2 or SEQ ID NO:3.

17. The method according to one of the preceding claims, wherein the OX1R agonist is an immunoadhesin.

18. The method according to one of the preceding claims, wherein the OX1R is an aptamer.

15 19. The method according to one of the preceding claims, wherein the pancreatic cancer is selected from the group consisting of pancreatic adenocarcinoma such as pancreatic ductal adenocarcinoma and other tumors of the exocrine pancreas such as serous cystadenomas, acinar cell cancers, intraductal papillary mucinous neoplasms (IPMN) and pancreatic neuroendocrine tumors such as insulinomas.

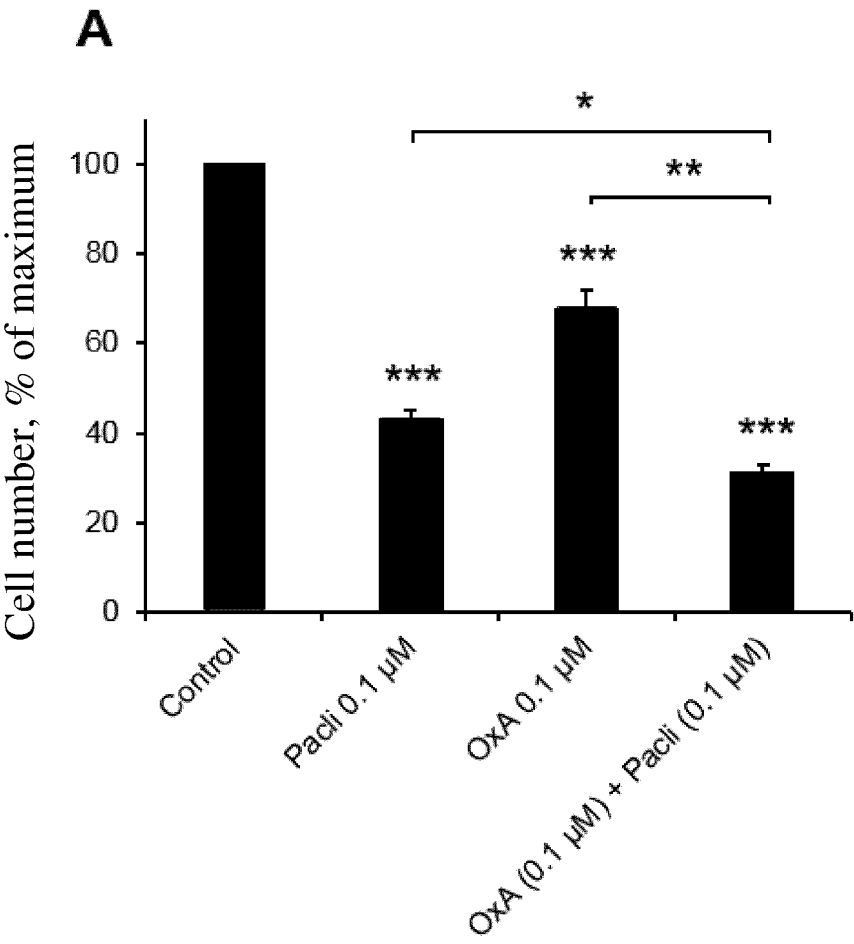


Figure 1A

2/5

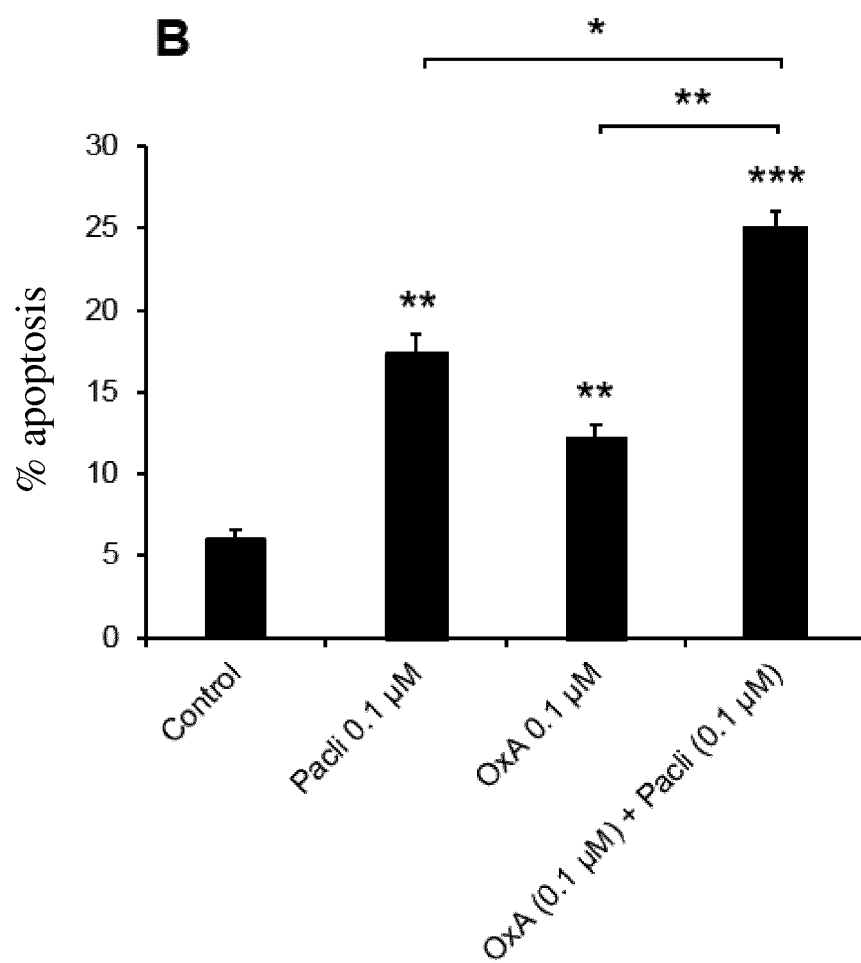


Figure 1B

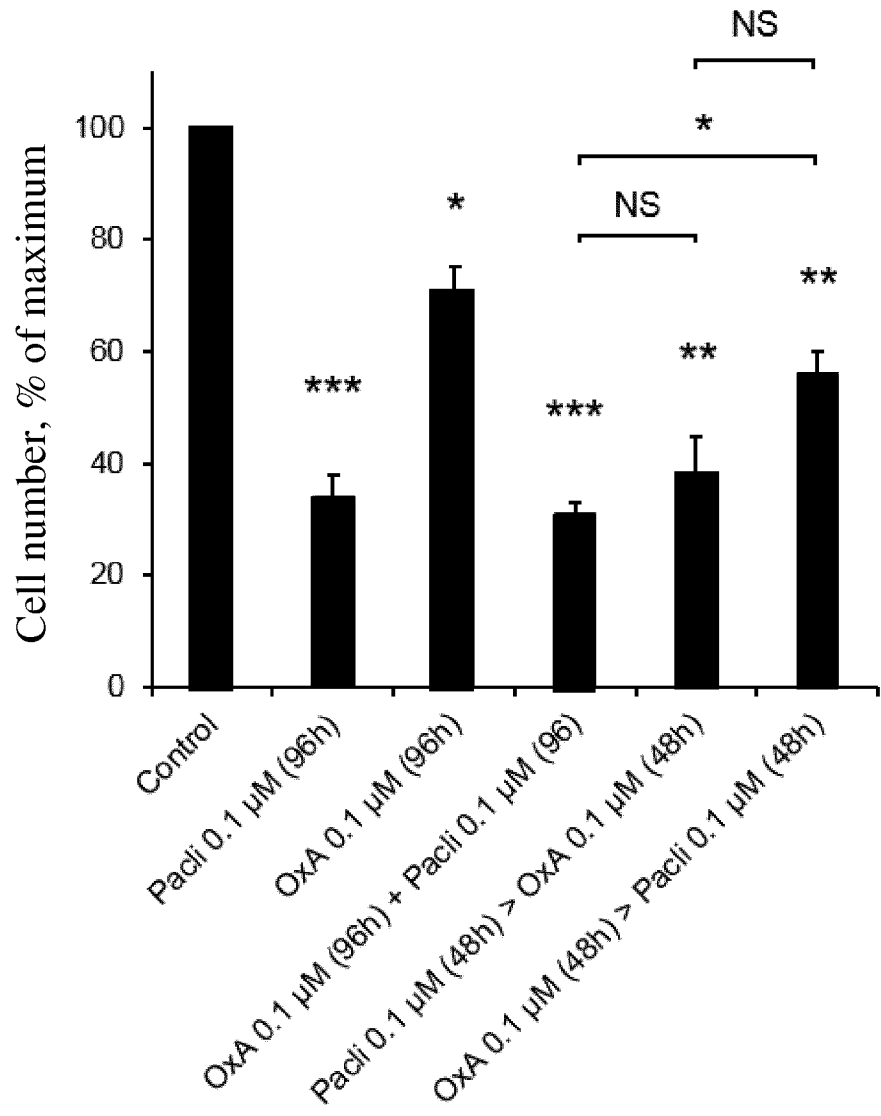


Figure 2

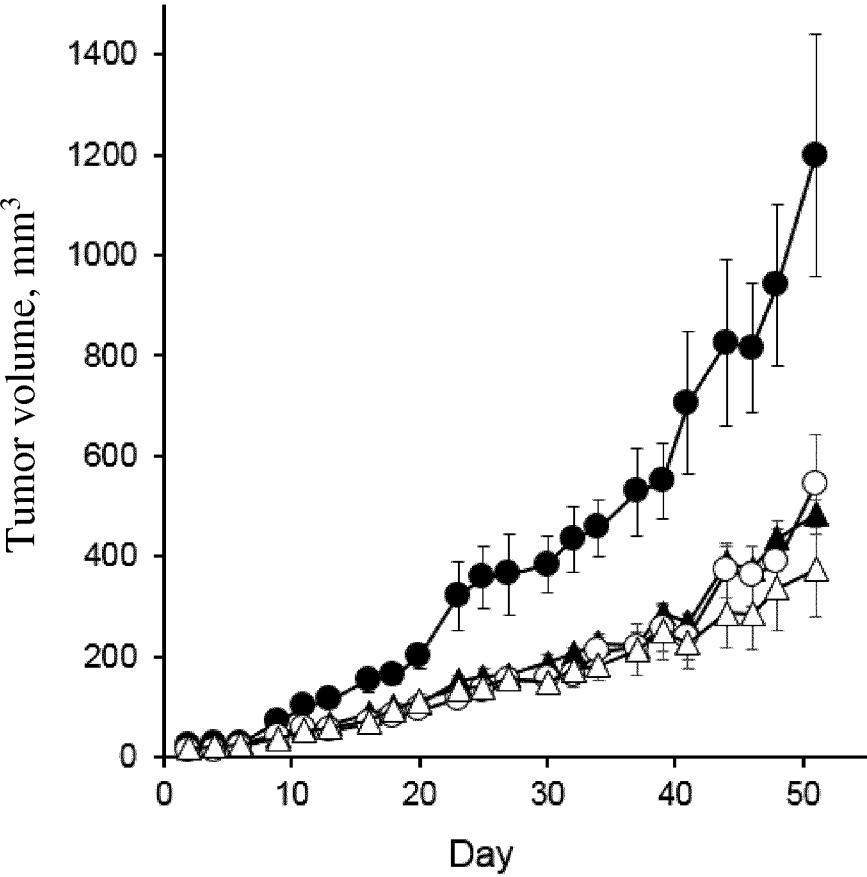


Figure 3

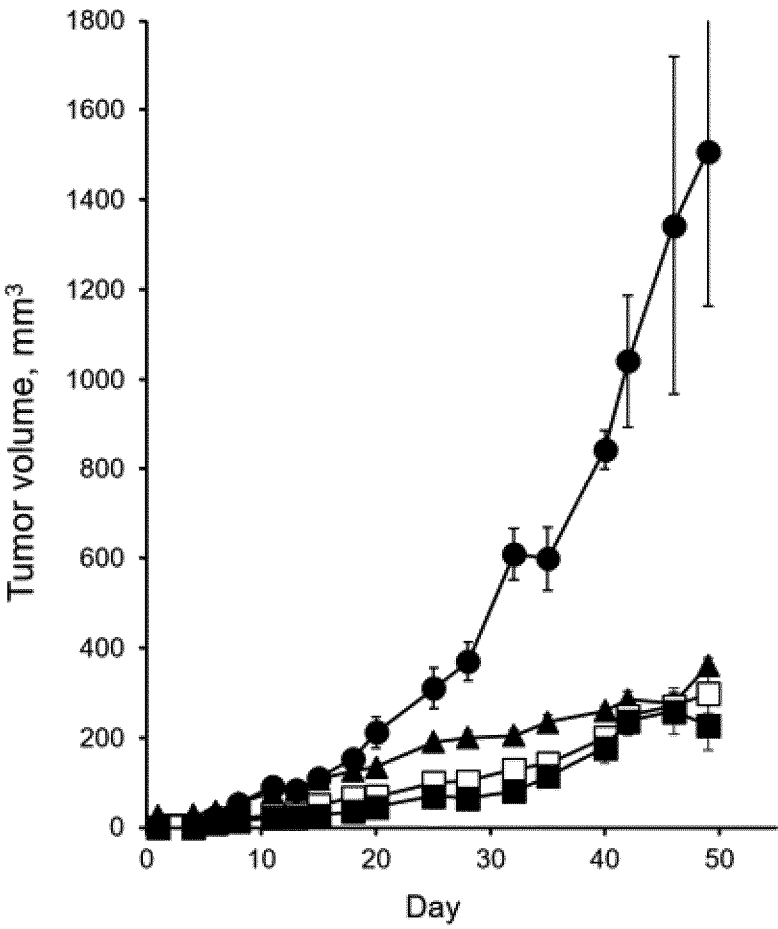


Figure 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/058499

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K38/22 A61K31/337 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

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, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DANIEL D. VON HOFF ET AL: "Increased Survival in Pancreatic Cancer with nab-Paclitaxel plus Gemcitabine", NEW ENGLAND JOURNAL OF MEDICINE, THE - NEJM -, vol. 369, no. 18, 31 October 2013 (2013-10-31), pages 1691-1703, XP055250743, ISSN: 0028-4793, DOI: 10.1056/NEJMoa1304369 abstract page 1699, right-hand column, paragraph 3 - page 1702, right-hand column, paragraph 3 ----- -/--	1-19



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

12 June 2017

Date of mailing of the international search report

22/06/2017

Name and mailing address of the ISA/

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Authorized officer

Bazzanini, Rita

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/058499

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2011/123393 A1 (ABRAXIS BIOSCIENCE LLC [US]; DESAI NEIL P [US]; SOON-SHIONG PATRICK [U] 6 October 2011 (2011-10-06) paragraphs [0014], [0026], [0029], [0030], [0086], [0164], [0219] - [0239] claims 1,2,4,9,10,13,14 -----	1-19
Y	WO 2015/071701 A1 (INSERM INST NAT DE LA SANTÉ ET DE LA RECH MÉDICALE [FR]; UNIV PARIS DI) 21 May 2015 (2015-05-21) cited in the application claims 1-13 page 22, paragraph 3 - page 23, paragraph 2 -----	1-19
A	KEN GARBER: "Stromal Depletion Goes on Trial in Pancreatic Cancer", JOURNAL OF THE NATIONAL CANCER INSTITUTE, vol. 102, no. 7, 7 April 2010 (2010-04-07), pages 448-450, XP055302304, cited in the application the whole document -----	1-19

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2017/058499

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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