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(54) Title: FUSION PROTEINS AND ANTIBODIES COMPRISING THEREOF FOR PROMOTING APOPTOSIS

(57) Abstract: The present invention relates to fusion proteins and antibodies comprising thereof for promoting apoptosis. In particular, the present invention relates to a fusion protein which comprises an immunoglobulin chain or a fragment thereof fused to an orexin polypeptide.



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FUSION PROTEINS AND ANTIBODIES COMPRISING THEREOF FOR PROMOTING APOPTOSIS

FIELD OF THE PRESENT INVENTION:

The present invention relates to fusion proteins and antibodies comprising thereof for promoting apoptosis.

BACKGROUND OF THE PRESENT INVENTION:

Monoclonal antibodies (mAb) are playing an increasing role in the management of many diseases and especially cancers. For instance, cetuximab is a recombinant, human/mouse chimeric monoclonal that competitively binds to the extracellular domain of EGFR with a higher affinity than its endogenous ligands, blocking EGFR-driven signalling, resulting in inhibition of cell growth and induction of apoptosis. There are also reports that cetuximab can mediate antibody-dependent cellular cytotoxicity against tumour cells. This monoclonal antibody has been proven effective in patients with KRAS wild-type metastatic colorectal cancer. In addition, there is evidence that not only do patients with KRAS mutations not benefit from treatment with cetuximab, but that it could actually have a detrimental effect on them. Another example is Trastuzumab (Herceptin) which is a humanized, monoclonal antibody that blocks the activity of HER2. Trastuzumab is the only anti-HER2 agent that is approved for adjuvant therapy in patients who have HER2-positive disease with either positive or negative lymph node status, estrogen receptor (ER)/progesterone receptor (PR)-negative disease, or a high-risk feature, either in combination with chemotherapy or as a single agent after chemotherapy. Response rates to single-agent trastuzumab range from 12 to 34% for metastatic breast cancer and significant improvements in survival rates are achieved in patients with early-stage HER2-overexpressing breast cancer in the adjuvant setting. However patients with a low expression of HER2 are not eligible for a treatment with Trastuzumab. So despite the considerable advances brought by monoclonal antibodies in the therapeutic management of cancers, there is still a place for improving the efficiency of monoclonal antibodies, notably for promoting apoptosis of cancer cells that are resistant to current monoclonal antibodies.

The orexins (hypocretins) comprise two neuropeptides produced in the hypothalamus: 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 more selective for OX-A than OX-B and the orexin-2 receptor (OX2R or HCTR2) binds 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). In particular, it was shown that OX1R promotes apoptosis in the cancer cell lines 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.). 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. Besides, expression of OX1R has been recently confirmed in pancreatic cancer, hepatocarcinomas, and advanced prostate cancer. Accordingly the prior art supports that OX1R is an Achilles's heel of cancers (even chemoresistance) and suggests that OX1R is a relevant target for cancer therapy.

SUMMARY OF THE PRESENT INVENTION:

The present invention relates to fusion proteins and antibodies comprising thereof for promoting apoptosis. In particular, the present invention is defined by the claims.

DETAILED DESCRIPTION OF THE PRESENT INVENTION:

5 The inventors surprisingly found that it is possible to fuse one orexin polypeptide to an immunoglobulin chain and that the antibody comprising said fusion protein is capable of promoting apoptosis of cancer cells.

10 Accordingly, the present invention relates to a fusion protein which comprises an immunoglobulin chain or a fragment thereof fused to an orexin polypeptide.

As used herein, the term “immunoglobulin chain” has its general meaning in the art and refers to the heavy or light immunoglobulin chains of an antibody. Actually in natural antibodies, two heavy chains are linked to each other by disulfide bonds and each heavy chain is linked to a light chain by a disulfide bond. There are two types of light chain, lambda (1) and kappa (k). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE. Each chain contains distinct sequence domains. The light chain includes two domains, a variable domain (VL) and a constant domain (CL). The heavy chain typically includes five domains, a variable domain (VH), three constant domains (CH1, CH2 and CH3, collectively referred to as CH) and a hinge domain which connects the CH1 domains to the CH2 domain with the exception of the IgM immunoglobulin which comprises 4 constant domains (CH1, CH2, CH3 and CH4). The variable regions of both light (VL) and heavy (VH) chains determine binding recognition and specificity to the antigen. The constant region domains of the light (CL) and heavy (CH) chains confer important biological properties such as antibody chain association, secretion, trans-placental mobility, complement binding, and binding to Fc receptors (FcR). The hinge region that links the Fc and Fab portions of the antibody molecule is in reality a flexible tether, allowing independent movement of the two Fab arms, rather than a rigid hinge. The Fv fragment is the N-terminal part of the Fab fragment of an immunoglobulin and consists of the variable portions of one light chain and one heavy chain. The specificity of the antibody resides in the structural complementarity between the antibody combining site and the antigenic determinant. Antibody combining sites are made up of residues that are primarily from the hypervariable or complementarity determining regions (CDRs). Occasionally, residues from nonhypervariable or framework regions (FR) can participate to the antibody

binding site or influence the overall domain structure and hence the combining site. Complementarity Determining Regions or CDRs refer to amino acid sequences which together define the binding affinity and specificity of the natural Fv region of a native immunoglobulin binding site. The light and heavy chains of an immunoglobulin each have three CDRs, designated L-CDR1, L-CDR2, L- CDR3 and H-CDR1, H-CDR2, H-CDR3, respectively. An antigen-binding site, therefore, typically includes six CDRs, comprising the CDR set from each of a heavy and a light chain V region. Framework Regions (FRs) refer to amino acid sequences interposed between CDRs. The residues in antibody variable domains are conventionally numbered according to a system devised by Kabat et al. This system is set forth in Kabat et al., 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA (hereafter "Kabat et al.").

In some embodiments, the immunoglobulin chain is a light immunoglobulin chain. In some embodiments, the immunoglobulin chain is a heavy immunoglobulin chain. In some embodiments, the heavy immunoglobulin chain is a heavy 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 single domain antibody are also called VHH or "nanobody®". For a general description of (single) domain antibodies, reference is also made to the prior art cited above, as well as to EP 0 368 684, Ward et al. (Nature 1989 Oct 12; 341 (6242): 544-6), Holt et al., Trends Biotechnol., 2003, 21(11):484-490; and WO 06/030220, WO 06/003388.

Accordingly, in some embodiments, the fusion protein of the present invention consists of an immunoglobulin light chain fused to an orexin polypeptide. In some embodiments, the fusion protein of the present invention consists of an immunoglobulin heavy chain fused to an orexin polypeptide.

In some embodiments, the fragment of an immunoglobulin chain comprises at least one constant domain of an immunoglobulin chain. In some embodiments, the constant domain is a CL domain which is from a lambda or a kappa light chain. In some embodiments, the constant domain is a CH1, CH2, CH3 or CH4 domains. In some embodiments, the CH domain is from an IgG, such as IgG1, IgG2, IgG3, or IgG4. In some embodiments, the CH domain is from an IgA, IgD, IgE or IgM. In some embodiments, the fragment of an immunoglobulin chain comprises a hinge region from an IgG, such as IgG1, IgG2, IgG3, or IgG4 or from an IgA, IgD, IgE or IgM.

In some embodiments, the fusion protein of the present invention consists of the VH-CH1 fragment of a heavy immunoglobulin chain fused to an orexin polypeptide. In some
embodiments, the fusion protein of the present invention consists of the VH-CH1-Hinge
5 fragment of a heavy immunoglobulin chain fused to an orexin polypeptide. In some
embodiments, the fusion protein of the present invention consists of the VH-CH1-Hinge-CH2
region fragment of a heavy immunoglobulin chain fused to an orexin polypeptide. In some
embodiments, the fusion protein of the present invention consists of the VH-CH1-Hinge-CH3
10 (“minibody”) region fragment of a heavy immunoglobulin chain fused to an orexin
polypeptide.

As used herein, the term “orexin polypeptide” refers to any polypeptide that is able to
bind to orexin receptor type 1 (OX1R) or orexin receptor type 2 (OX2R) and that is able to
promote apoptosis. As used herein the term “OX1R” has its general meaning in the art and
15 refers to orexin receptor type 1, also known as hypocretin receptor type 1, which is a protein
that in humans is encoded by the HCRTR1 gene. As used herein the term “OX2R” has its
general meaning in the art and refers to orexin receptor type2, also known as hypocretin
receptor type 2, which is a protein that in humans is encoded by the HCRTR2 gene. In
particular, OX1R promotes apoptosis in the various cancer cell lines through a mechanism
20 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
25 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
30 Dec;23(12):4069-80. doi: 10.1096/fj.09-131367. Epub 2009 Aug 6.). Human antibodies of the
present invention are thought to be capable of promoting apoptosis of cancer cells via the
same mechanism. As used herein the term “orexin” has its general meaning in the art and
include orexin A and orexin B. An exemplary human amino acid sequence for Orexin A is
SEQ ID NO:1 and an exemplary human amino acid sequence for Orexin B is SEQ ID NO:2.

Orexin-A (SEQ ID NO:1): p EPLPDCCRQKTCSCRLYELLHGAGNHAAGILTL
wherein p e means pyroglutamate

5 Orexin-B (SEQ ID NO:2): RSGPPGLQGRLQRLQASGNHAAGILTM

10 In some embodiments, the orexin polypeptide of the present invention has at least 50%, preferably at least 60%, more preferably at least 80%, still more preferably at least 95% of identity with SEQ ID NO: 1 or SEQ ID NO: 2. According to the present invention a first amino acid sequence having at least 50% of identity with a second amino acid sequence means that the first sequence has 50; 51; 52; 53; 54; 55; 56; 57; 58; 59; 60; 61; 62; 63; 64; 65; 66; 67; 68; 69; 70; 71; 72; 73; 74; 75; 76; 77; 78; 79; 80; 81; 82; 83; 84; 85; 86; 87; 88; 89; 90; 91; 92; 93; 94; 95; 96; 97; 98; 99; or 100% of identity with the second amino acid sequence. According to the present invention a first amino acid sequence having at least 90%
15 of identity with a second amino acid sequence means that the first sequence has 90; 91; 92; 93; 94; 95; 96; 97; 98; 99; or 100% of identity with the second amino acid sequence. According to the present invention a first amino acid sequence having at least 95% of identity with a second amino acid sequence means that the first sequence has 95; 96; 97; 98; 99; or 100% of identity with the second amino acid sequence.

20

 In some embodiments, the orexin polypeptide is SEQ ID NO:1.

 In some embodiments, the orexin polypeptide is SEQ ID NO:13.

25 In some embodiments, the orexin polypeptide 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:2 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.

30 In some embodiments, the orexin polypeptide comprises the amino acid sequence ranging from the amino acid residue at position 10 to the amino acid residue at position 28 in SEQ ID NO:2 wherein at least one amino acid residue position 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.

5 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
10 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

15 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

20 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 hydrophobic/hydrophilic properties and residue weight/size also is substantially retained in the polypeptide of the present invention as compared to the native
25 sequence of Orxin-B. The importance of the hydrophobic amino acid index in conferring interactive biologic function on a protein is generally understood in the art. It is accepted that the relative hydrophobic 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
30 amino acid has been assigned a hydrophobic 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); tryptophan (-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).

5 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:2.

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

In some embodiments, the orexin polypeptide is SEQ ID NO:2.

15 In some embodiments, the orexin polypeptide is SEQ ID NO:14.

SEQ ID NO: 14:

GLQGRLQRLQLQASGNHAAGILTM

20 In some embodiments, the last methionine residue in SEQ ID NO:2 or SEQ ID NO:14 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.

25 In some embodiments, the orexin polypeptide is extended at its c-terminal end by at least one amino acid. In some embodiments, the orexin polypeptide is extended at its c-terminal end by at least one glycine (G). In some embodiments, the orexin polypeptide is extended at its c-terminal end by at least 2 amino acids. In some embodiments, the orexin polypeptide is extended at its c-terminal end by the amino acid sequence GR or GK. In some embodiments, the orexin polypeptide is extended at its c-terminal end by at least 3 amino acids. In some embodiments, the orexin polypeptide is extended at its c-terminal end by the amino acid sequence GRR, GRK, GKR, or GKK. In said embodiments, the methionine residue at position 28 is not necessarily amidated.

In some embodiments, the orexin polypeptide comprises or consists of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12.

In some embodiment, the immunoglobulin chain of the fusion protein of the present invention is fused at its C-terminal end to the N-terminal end of the orexin polypeptide.

5 In some embodiment, the immunoglobulin chain of the fusion protein of the present invention is fused at its N-terminal end to the C-terminal end of the orexin polypeptide extended by 1, 2, or 3 amino acids as described above. In some embodiment, the immunoglobulin chain of the fusion protein of the present invention is fused at its N-terminal end to the C-terminal end of the orexin polypeptide extended by the amino acid sequence
10 GRR, GRK, GKR, or GKK.

In some embodiments, the immunoglobulin chain and the orexin polypeptide are fused to each other directly (i.e. without use of a linker) or via a linker. Suitable linkers will be clear to the skilled person based on the disclosure herein, optionally after some limited degree of
15 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.
20 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
25 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)3.

In some embodiments, the fusion protein of the present invention consists of the amino
30 acid sequence as set forth in SEQ ID NO:3:

SEQ ID NO:3: Cetuximab L kappa fused to Orexin B

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESIS
GIPSRFSGSGSGTDFTLINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPS

VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS
TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECRSGPPGLQGRLQRL
LQASGNHAAGILTM

5 In some embodiments, the last methionine residue of SEQ ID NO: 3 is amidated.

The fusion protein of the present invention may be produced by any technique known in the art, such as, without limitation, any chemical, biological, genetic or enzymatic technique, either alone or in combination. For example, knowing the amino acid sequence of the desired sequence, one skilled in the art can readily produce said fusion protein, by standard techniques for production of polypeptides. For instance, they can be synthesized using well-known solid phase method, preferably using a commercially available peptide synthesis apparatus (such as that made by Applied Biosystems, Foster City, California) and following the manufacturer's instructions. Alternatively, the fusion protein of the present invention can be synthesized by recombinant DNA techniques well-known in the art. For example, the fusion protein of the present invention can be obtained as DNA expression products after incorporation of DNA sequences encoding the fusion protein into expression vectors and introduction of such vectors into suitable eukaryotic or prokaryotic hosts that will express the desired fusion protein, from which they can be later isolated using well-known techniques.

Accordingly, a further object of the present invention relates to a nucleic acid molecule encoding a fusion protein of the present invention, which thus comprises a first nucleic acid sequence encoding for the immunoglobulin chain or the fragment thereof operably linked to a second nucleic acid sequence encoding for the orexin polypeptide.

As used herein, the term "operably linked" refers to a linkage of polynucleotide elements in a functional relationship. A nucleic acid sequence is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. Operably linked means that the DNA sequences being linked are typically contiguous.

Typically, said nucleic acid molecule is a DNA or RNA molecule, which may be included in any suitable vector. The term "vector," as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked.

One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (for instance
5 bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (such as non-episomal mammalian vectors) may be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant
10 expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the present invention is intended to include such other forms of expression vectors, such as viral vectors (such as replication-defective
15 retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

So, a further object of the present invention relates to a vector comprising a nucleic acid of the present invention.

20 Such vectors may comprise regulatory elements, such as a promoter, enhancer, terminator and the like, to cause or direct expression of said antibody upon administration to a subject. Examples of promoters and enhancers used in the expression vector for animal cell include early promoter and enhancer of SV40 (Mizukami T. et al. 1987), LTR promoter and enhancer of Moloney mouse leukemia virus (Kuwana Y et al. 1987), promoter (Mason JO et
25 al. 1985) and enhancer (Gillies SD et al. 1983) of immunoglobulin H chain and the like. Any expression vector for animal cell can be used, so long as a gene encoding the human antibody C region can be inserted and expressed. Examples of suitable vectors include pAGE107 (Miyaji H et al. 1990), pAGE103 (Mizukami T et al. 1987), pHSG274 (Brady G et al. 1984), pKCR (O'Hare K et al. 1981), pSG1 beta d2-4-(Miyaji H et al. 1990) and the like. Other
30 examples of plasmids include replicating plasmids comprising an origin of replication, or integrative plasmids, such as for instance pUC, pcDNA, pBR, and the like. Other examples of viral vector include adenoviral, retroviral, herpes virus and AAV vectors. Such recombinant viruses may be produced by techniques known in the art, such as by transfecting packaging cells or by transient transfection with helper plasmids or viruses. Typical examples of virus

packaging cells include PA317 cells, PsiCRIP cells, GPenv+ cells, 293 cells, etc. Detailed protocols for producing such replication-defective recombinant viruses may be found for instance in WO 95/14785, WO 96/22378, US 5,882,877, US 6,013,516, US 4,861,719, US 5,278,056 and WO 94/19478.

5

A further object of the present invention relates to a host cell which has been transfected, infected or transformed by a nucleic acid and/or a vector according to the present invention.

10

The term "transformation" means the introduction of a "foreign" (i.e. extrinsic or extracellular) gene, DNA or RNA sequence to a host cell, so that the host cell will express the introduced gene or sequence to produce a desired substance, typically a protein or enzyme coded by the introduced gene or sequence. A host cell that receives and expresses introduced DNA or RNA has been "transformed".

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The nucleic acids of the present invention may be used to produce a fusion protein of the present invention in a suitable expression system. The term "expression system" means a host cell and compatible vector under suitable conditions, e.g. for the expression of a protein coded for by foreign DNA carried by the vector and introduced to the host cell. Common expression systems include E. coli host cells and plasmid vectors, insect host cells and Baculo virus vectors, and mammalian host cells and vectors. Other examples of host cells include, without limitation, prokaryotic cells (such as bacteria) and eukaryotic cells (such as yeast cells, mammalian cells, insect cells, plant cells, etc.). Specific examples include E.coli, Kluyveromyces or Saccharomyces yeasts, mammalian cell lines (e.g., Vero cells, CHO cells, 3T3 cells, COS cells, etc.) as well as primary or established mammalian cell cultures (e.g., produced from lymphoblasts, fibroblasts, embryonic cells, epithelial cells, nervous cells, adipocytes, etc.). Examples also include mouse SP2/0-Ag14 cell (ATCC CRL1581), mouse P3X63-Ag8.653 cell (ATCC CRL1580), CHO cell in which a dihydrofolate reductase gene (hereinafter referred to as "DHFR gene") is defective (Urlaub G et al; 1980), rat YB2/3HL.P2.G1 1.16Ag.20 cell (ATCC CRL1662, hereinafter referred to as "YB2/0 cell"), and the like.

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The present invention also relates to a method of producing a recombinant host cell expressing a fusion protein of the present invention, said method comprising the steps of: (i)

introducing in vitro or ex vivo a recombinant nucleic acid or a vector as described above into a competent host cell, (ii) culturing in vitro or ex vivo the recombinant host cell obtained and (iii), optionally, selecting the cells which express and/or secrete said fusion protein. Such recombinant host cells can be used for the production of the fusion protein of the present invention.

Methods for producing amidated polypeptide are well known in the art and typically involve use of amidation enzyme. As used herein, the term “amidation enzyme” is defined as the enzymes which can convert the carboxyl group of a polypeptide to an amide group. Enzymes capable of C-terminal amidation of peptides have been known for a long time (Eipper et al. Mol. Endocrinol. 1987 November; 1 (11): 777). Examples of amidating enzymes include peptidylglycine α -monooxygenase (EC 1.14.17.3), herein referred to as PAM, and peptidylamidoglycolate lyase (EC 4.3.2.5), herein referred to as PGL. The preparation and purification of such PAM enzymes is familiar to the skilled worker and has been described in detail (M. Nogudi et al. Prot. Expr. Purif. 2003, 28: 293). An alternative to the “in vitro” amidation by means of PAM emerges when the enzyme is coexpressed in the same host cell with the precursor protein to be amidated (i.e the fusion protein of the present invention). This is achieved by introducing a gene sequence which codes for a PAM activity into the host cell under the control of a host-specific regulatory sequence. This expression sequence can either be incorporated stably into the respective chromosomal DNA sequence, or be present on a second plasmid parallel to the expression plasmid for the target protein (i.e. fusion protein of the present invention), or be integrated as second expression cassette on the same vector, or be cloned in a polycistronic expression approach in phase with the gene sequence which encodes the target protein (i.e. fusion protein of the present invention) under the control of the same promoter sequence. A further method for amidation is based on the use of protein-specific self-cleavage mechanisms (Cottingham et al. Nature Biotech. Vol. 19, 974-977, 2001). The amidation processes described above start from a C terminus of the target peptide which is extended by at least one amino acid glycine or alternatively interim peptide. Alternative methods, are also described in WO2007036299.

Accordingly, in some embodiments, the nucleic acid sequence encoding for the orexin polypeptide is chosen to allow the amidation of said orexin polypeptide and thus may comprise additional codons that will code for a glycine-extended precursor. Typically, the glycine-extended precursor resembles YGXX, where Y represents the amino acid that shall be

amidated and X represents any amino acid so that the amidation enzyme (e.g. PAM) catalyzes the production of the amidated polypeptide from said glycine-extended precursor. In some embodiments, the glycine-extended precursor is MG, MGR, MGRR, MGK or MGKK.

5 A further object of the present invention relates to an antibody which comprises at least one fusion protein of the present invention.

As used herein the term "antibody" or "immunoglobulin" have the same meaning, and will be used equally in the present invention. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. As such, the term antibody encompasses not only whole antibody molecules, but also antibody fragments as well as variants (including derivatives) of antibodies and antibody fragments. In particular, the term "antibody" is thus used to refer to any antibody-like molecule that has an antigen binding region, and this term includes antibody fragments that comprise an antigen binding domain such as Fab', Fab, F(ab')₂, single domain antibodies (DABs or VHH), TandAbs dimer, Fv, scFv (single chain Fv), dsFv, ds-scFv, Fd, linear antibodies, minibodies, diabodies, bispecific antibody fragments, bibody, tribody (scFv-Fab fusions, bispecific or trispecific, respectively); sc-diabody; kappa(lamda) bodies (scFv-CL fusions); DVD-Ig (dual variable domain antibody, bispecific format); SIP (small immunoprotein, a kind of minibody); SMIP ("small modular immunopharmaceutical" scFv-Fc dimer; DART (ds-stabilized diabody "Dual Affinity ReTargeting"); small antibody mimetics comprising one or more CDRs and the like. The techniques for preparing and using various antibody-based constructs and fragments are well known in the art.

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In some embodiments, the antibody of the present invention is a monoclonal antibody. In some embodiments, the antibody of the present invention is a chimeric antibody. In some embodiments, the antibody of the present invention is a humanized antibody.

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As used herein the term "chimeric antibody" refers to an antibody which comprises a VH domain and a VL domain of an antibody derived from a non human antibody (e.g. murine antibody), and a CH domain and a CL domain of a human antibody. As used herein, the term "humanized antibody" refers to an antibody having variable region framework and constant

regions from a human antibody but retains the CDRs of a non human antibody (e.g. murine antibody).

The antibody of the present invention may be of any isotype. The choice of isotype typically will be guided by the desired effector functions, such as ADCC induction. Exemplary isotypes are IgG1, IgG2, IgG3, and IgG4. Either of the human light chain constant regions, kappa or lambda, may be used. If desired, the class of a monoclonal antibody of the present invention may be switched by known methods. Typical, class switching techniques may be used to convert one IgG subclass to another, for instance from IgG1 to IgG2. Thus, the effector function of the monoclonal antibodies of the present invention may be changed by isotype switching to, e.g., an IgG1, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM antibody for various therapeutic uses. In some embodiments, the antibody of the present invention is a full-length antibody. In some embodiments, the full-length antibody is an IgG1 antibody. In some embodiments, the full-length antibody is an IgG4 antibody. In some embodiments, the specific IgG4 antibody is a stabilized IgG4 antibody. Examples of suitable stabilized IgG4 antibodies are antibodies wherein arginine at position 409 in a heavy chain constant region of human IgG4, which is indicated in the EU index as in Kabat et al. supra, is substituted with lysine, threonine, methionine, or leucine, preferably lysine (described in WO2006033386) and/or wherein the hinge region comprises a Cys-Pro-Pro-Cys sequence. Other suitable stabilized IgG4 antibodies are disclosed in WO2008145142, which is hereby incorporated by reference in its entirety. In some embodiments, the monoclonal antibody of the present invention is an antibody of a non-IgG4 type, e.g. IgG1, IgG2 or IgG3 which has been mutated such that the ability to mediate effector functions, such as ADCC, has been reduced or even eliminated. Such mutations have e.g. been described in Dall'Acqua WF et al., J Immunol. 177(2) : 1129-1138 (2006) and Hezareh M, J Virol. 75(24) : 12161-12168 (2001).

In some embodiments, the antibody is an antigen-binding fragment. Antibody fragments can be obtained by conventional techniques, such as by fragmentation of full-length antibodies or by expression of nucleic acids encoding antibody fragments in recombinant cells (see, for instance Evans et al., J. Immunol. Meth. 184, 123-38 (1995)). The fragments can then be tested or screened for their properties in the same manner as described herein for full-length antibodies.

In some embodiments, the antibody fragment of the present invention is a F(ab')₂ fragment, which is a bivalent fragments comprising two Fab fragments linked by a disulfide bridge at the hinge region. These can be generated by, e.g., treating a full-length antibody with pepsin.

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In some embodiments, the antibody fragment of the present invention is a Fab' or Fab fragment, which is a monovalent fragment consisting of the VL, VH, CL and CH1 domains. Fab fragments can be obtained, e.g., by treating an IgG antibody with papain. Fab' fragments can be obtained, e.g., by reducing the disulfide bridges of a F(ab')₂ fragment using a reducing agent such as dithiothreitol.

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In some embodiments, the antibody fragment of the present invention is a Fd fragments, which consist essentially of the VH and CH1 domains.

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In some embodiments, the antibody fragment of the present invention is a Fv fragment, which consists essentially of the VL and VH domains of a single arm of an antibody and single-chain antibodies thereof. Single-chain antibodies (also known as single chain Fv (scFv) antibodies) are constructs where the VL and VH domains of an Fv fragment are joined, using recombinant methods, by a synthetic linker that enables them to be expressed as a single protein chain in which the VL and VH regions pair to form monovalent molecules (see for instance Bird et al., Science 242, 423-426 (1988) and Huston et al., PNAS USA 85, 5879-5883 (1988)).

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In some embodiments, the antibody fragment is a Fc fragment.

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In some embodiments, the antibody of the present invention comprises at least one fusion protein of the present invention so that the C-terminal end of the orexin polypeptide is free (i.e. not fused to any third polypeptide or linked to a further group (e.g. a toxin) or fused to a glycine-extension precursor.

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Depending on the format, the antibody of the present invention may thus comprise 1, 2, 3, or 4 orexin polypeptide.

In some embodiments, a full-length antibody of the present invention comprises:

- one immunoglobulin chain (i.e. a light or a heavy chain) fused to an orexin polypeptide,
- two immunoglobulin chains (i.e. the 2 light chains, the 2 heavy chains, or one light chain and one heavy chain) fused to an orexin polypeptide,
- 5 - three immunoglobulin chains (i.e. the 2 light chains and one heavy chain, 2 heavy chains and one light chain fused to an orexin polypeptide) or
- four immunoglobulin chains (i.e. the 2 light chains and the 2 heavy chains) fused to an orexin polypeptide.

10 In some embodiments, a F(ab')₂ fragment of the present invention comprises:

- one immunoglobulin chain (i.e. a light or a VH-CH1-Hinge chain) fused to an orexin polypeptide,
- two immunoglobulin chains (i.e. the 2 light chains, the 2 VH-CH1-Hinge chains, or one light chain and one VH-CH1-Hinge chain) fused to an orexin polypeptide,
- 15 - three immunoglobulin chains (i.e. the 2 light chains and one VH-CH1-Hinge chain, 2 VH-CH1-Hinge chains and one light chain fused to an orexin polypeptide) or
- four immunoglobulin chains (i.e. the 2 light chains and the 2 VH-CH1-Hinge chains) fused to an orexin polypeptide.

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In some embodiments, a Fab fragment of the present invention comprises:

- one immunoglobulin chain (i.e. a light or a VH-CH1 chain) fused to an orexin polypeptide, or
- two immunoglobulin chains (i.e. the light chain and the VH-CH1 chain) fused to
- 25 an orexin polypeptide,

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In addition or alternative to modifications made within the framework or CDR regions, antibodies of the present invention may be engineered to include modifications within the Fc region, typically to alter one or more functional properties of the antibody, such as serum half-life, complement fixation, Fc receptor binding, and/or antigen-dependent cellular cytotoxicity. Furthermore, a monoclonal antibody of the present invention may be chemically modified (e.g., one or more chemical moieties can be attached to the antibody) or be modified to alter its glycosylation, again to alter one or more functional properties of the antibody. For example, it will be appreciated that the affinity of antibodies provided by the

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present invention may be altered using any suitable method known in the art. The present invention therefore also relates to variants of the antibody molecules of the present invention, which have an improved affinity for their antigen. Such variants can be obtained by a number of affinity maturation protocols including mutating the CDRs (Yang et al., J. Mol. Biol., 254, 392-403, 1995), chain shuffling (Marks et al., Bio/Technology, 10, 779-783, 1992), use of mutator strains of E. coli (Low et al., J. Mol. Biol., 250, 359-368, 1996), DNA shuffling (Patten et al., Curr. Opin. Biotechnol., 8, 724-733, 1997), phage display (Thompson et al., J. Mol. Biol., 256, 77-88, 1996) and sexual PCR (Cramer et al., Nature, 391, 288-291, 1998). Vaughan et al. (supra) discusses these methods of affinity maturation.

In some embodiments, the hinge region of CH1 is modified such that the number of cysteine residues in the hinge region is altered, e.g., increased or decreased. This approach is described further in U.S. Patent No. 5,677,425 by Bodmer et al. The number of cysteine residues in the hinge region of CH1 is altered to, for example, facilitate assembly of the light and heavy chains or to increase or decrease the stability of the antibody.

In some embodiments, the monoclonal antibody of the present invention is modified to increase its biological half-life. Various approaches are possible. For example, one or more of the following mutations can be introduced: T252L, T254S, T256F, as described in U.S. Patent No. 6,277,375 by Ward. Alternatively, to increase the biological half-life, the antibody can be altered within the CH1 or CL region to contain a salvage receptor binding epitope taken from two loops of a CH2 domain of an Fc region of an IgG, as described in U.S. Patent Nos. 5,869,046 and 6,121,022 by Presta et al.

In some embodiments, the Fc region is altered by replacing at least one amino acid residue with a different amino acid residue to alter the effector functions of the antibody. For example, one or more amino acids can be replaced with a different amino acid residue such that the antibody has an altered affinity for an effector ligand but retains the antigen-binding ability of the parent antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in further detail in U.S. Patent Nos. 5,624,821 and 5,648,260, both by Winter et al.

In some embodiments, one or more amino acids selected from amino acid residues can be replaced with a different amino acid residue such that the antibody has altered C1q binding

and/or reduced or abolished complement dependent cytotoxicity (CDC). This approach is described in further detail in U.S. Patent Nos. 6,194,551 by Idusogie et al.

In some embodiments, one or more amino acid residues are altered to thereby alter the ability of the antibody to fix complement. This approach is described further in PCT Publication WO 94/29351 by Bodmer et al. In some embodiments, the Fc region is modified to increase the ability of the antibody to mediate antibody dependent cellular cytotoxicity (ADCC) and/or to increase the affinity of the antibody for an Fc receptor by modifying one or more amino acids. This approach is described further in PCT Publication WO 00/42072 by Presta. Moreover, the binding sites on human IgG1 for FcγRI, FcγRII, FcγRIII and FcRn have been mapped and variants with improved binding have been described (see Shields, R. L. et al, 2001 J. Biol. Chem. 276:6591-6604, WO2010106180).

In some embodiments, the glycosylation of an antibody is modified. For example, an aglycosylated antibody can be made (i.e., the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for the antigen. Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region framework glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation may increase the affinity of the antibody for antigen. Such an approach is described in further detail in U.S. Patent Nos. 5,714,350 and 6,350,861 by Co et al. Additionally or alternatively, an antibody can be made that has an altered type of glycosylation, such as a hypofucosylated or non-fucosylated antibody having reduced amounts of or no fucosyl residues or an antibody having increased bisecting GlcNAc structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant antibodies of the present invention to thereby produce an antibody with altered glycosylation. For example, EP 1,176,195 by Hang et al. describes a cell line with a functionally disrupted FUT8 gene, which encodes a fucosyl transferase, such that antibodies expressed in such a cell line exhibit hypofucosylation or are devoid of fucosyl residues. Therefore, in some embodiments, the monoclonal antibodies of the present invention may be produced by recombinant expression

in a cell line which exhibit hypofucosylation or non-fucosylation pattern, for example, a mammalian cell line with deficient expression of the FUT8 gene encoding fucosyltransferase. PCT Publication WO 03/035835 by Presta describes a variant CHO cell line, Lec13 cells, with reduced ability to attach fucose to Asn(297)-linked carbohydrates, also resulting in hypofucosylation of antibodies expressed in that host cell (see also Shields, R.L. et al, 2002 J. Biol. Chem. 277:26733-26740). PCT Publication WO 99/54342 by Umana et al. describes cell lines engineered to express glycoprotein-modifying glycosyl transferases (e.g., beta(1,4)-N acetylglucosaminyltransferase III (GnTIII)) such that antibodies expressed in the engineered cell lines exhibit increased bisecting GlcNAc structures which results in increased ADCC activity of the antibodies (see also Umana et al, 1999 Nat. Biotech. 17: 176-180). Eureka Therapeutics further describes genetically engineered CHO mammalian cells capable of producing antibodies with altered mammalian glycosylation pattern devoid of fucosyl residues (<http://www.eurekainc.com/a&boutus/companyoverview.html>). Alternatively, the monoclonal antibodies of the present invention can be produced in yeasts or filamentous fungi engineered for mammalian- like glycosylation pattern and capable of producing antibodies lacking fucose as glycosylation pattern (see for example EP1297172B1).

Another modification of the antibodies herein that is contemplated by the present invention is pegylation. An antibody can be pegylated to, for example, increase the biological (e.g., serum) half-life of the antibody. To pegylate an antibody, the antibody, or fragment thereof, typically is reacted with polyethylene glycol (PEG), such as a reactive ester or aldehyde derivative of PEG, under conditions in which one or more PEG groups become attached to the antibody or antibody fragment. The pegylation can be carried out by an acylation reaction or an alkylation reaction with a reactive PEG molecule (or an analogous reactive water-soluble polymer). As used herein, the term "polyethylene glycol" is intended to encompass any of the forms of PEG that have been used to derivatize other proteins, such as mono (CI- CIO) alkoxy- or aryloxy-poly ethylene glycol or polyethylene glycol-maleimide. In some embodiments, the antibody to be pegylated is an aglycosylated antibody. Methods for pegylating proteins are known in the art and can be applied to the monoclonal antibodies of the present invention. See for example, EP O 154 316 by Nishimura et al. and EP 0 401 384 by Ishikawa et al.

Another modification of the antibodies that is contemplated by the present invention is a conjugate or a protein fusion of at least the antigen-binding region of the monoclonal

antibody of the present invention to serum protein, such as human serum albumin or a fragment thereof to increase half-life of the resulting molecule. Such approach is for example described in Ballance et al. EP0322094.

5 The antibody of the present invention may be produced by any technique known in the art, such as, without limitation, any chemical, biological, genetic or enzymatic technique, either alone or in combination. Typically, the antibody of the present invention can be synthesized by recombinant DNA techniques well-known in the art. For example, the antibody of the present invention can be obtained as DNA expression products after
10 incorporation of DNA sequences encoding for the immunoglobulin chains or fragments thereof fused or not to the orexin polypeptide into expression vectors and introduction of such vectors into suitable eukaryotic or prokaryotic hosts that will express the desired antibody, from which they can be later isolated using well-known techniques.

15 The antibody of the present invention is directed against any antigen.

For example, the antibody of the present invention may be specific for an immune cell regulatory molecule such as CD3, CD4, CD8, CD25, CD28, CD26, CTLA-4, ICOS, or CD11a. Other suitable antigens include but are not limited to those associated with immune
20 cells including T cell-associated molecules, such as TCR/CD3 or CD2; NK cell-associated targets such as NKG2D, FcγRIIIa (CD16), CD38, CD44, CD56, or CD69; granulocyte-associated targets such as FcγRI (CD64), FcγRI (CD89), and CR3 (CD11b/CD18); monocyte/macrophage-associated targets (such as FcγRI (CD64), FcγRI (CD89), CD3 (CD11b/CD18), or mannose receptor; dendritic cell-associated targets such as FcγRI (CD64)
25 or mannose receptor; and erythrocyte-associated targets such as CRI (CD35).

Alternatively, the antibody of the present invention may be directed against a cancer antigen. Known cancer antigens include, without limitation, c-erbB-2 (erbB-2 is also known as c-neu or HER-2), which is particularly associated with breast, ovarian, and colon tumor
30 cells, as well as neuroblastoma, lung cancer, thyroid cancer, pancreatic cancer, prostate cancer, renal cancer and cancers of the digestive tract. Another class of cancer antigens is oncofetal proteins of nonenzymatic function. These antigens are found in a variety of neoplasms, and are often referred to as "tumor-associated antigens." Carcinoembryonic antigen (CEA), and alpha-fetoprotein (AFP) are two examples of such cancer antigens. AFP

levels rise in patients with hepatocellular carcinoma: 69% of patients with liver cancer express high levels of AFP in their serum. CEA is a serum glycoprotein of 200 kDa found in adenocarcinoma of colon, as well as cancers of the lung and genitourinary tract. Yet another class of cancer antigens is those antigens unique to a particular tumor, referred to sometimes as "tumor specific antigens," such as heat shock proteins (e.g., hsp70 or hsp90 proteins) from a particular type of tumor. Other targets include the MICA/B ligands of NKG2D. These molecules are expressed on many types of tumors, but not normally on healthy cells. Additional specific examples of cancer antigens include epithelial cell adhesion molecule (Ep-CAM/TACSTD1), mesothelin, tumor-associated glycoprotein 72 (TAG-72), gp100, Melan-A, MART-1, KDR, RCAS1, MDA7, cancer-associated viral vaccines (e.g., human papillomavirus antigens), prostate specific antigen (PSA, PSMA), RAGE (renal antigen), CAMEL (CTL-recognized antigen on melanoma), CT antigens (such as MAGE-B5, -B6, -C2, -C3, and D; Mage-12; CT10; NY-ESO-1, SSX-2, GAGE, BAGE, MAGE, and SAGE), mucin antigens (e.g., MUC1, mucin-CA125, etc.), cancer-associated ganglioside antigens, tyrosinase, gp75, C-myc, Mart1, MelanA, MUM-1, MUM-2, MUM-3, HLA-B7, Ep-CAM, tumor-derived heat shock proteins, and the like (see also, e.g., Acres et al., *Curr Opin Mol Ther* 2004 February, 6:40-7; Taylor-Papadimitriou et al., *Biochim Biophys Acta*. 1999 Oct. 8; 1455(2-3):301-13; Emens et al., *Cancer Biol Ther*. 2003 July-August; 2(4 Suppl 1):S161-8; and Ohshima et al., *Int J Cancer*. 2001 Jul. 1; 93(1):91-6). Other exemplary cancer antigen targets include CA 195 tumor-associated antigen-like antigen (see, e.g., U.S. Pat. No. 5,324,822) and female urine squamous cell carcinoma-like antigens (see, e.g., U.S. Pat. No. 5,306,811), and the breast cell cancer antigens described in U.S. Pat. No. 4,960,716. The antibody of the present invention may target protein antigens, carbohydrate antigens, or glycosylated proteins. For example, the variable domain can target glycosylation groups of antigens that are preferentially produced by transformed (neoplastic or cancerous) cells, infected cells, and the like (cells associated with other immune system-related disorders). In one aspect, the antigen is a tumor-associated antigen. In an exemplary aspect, the antigen is O-acetylated-GD2 or glypican-3. In another particular aspect, the antigen is one of the Thomsen-Friedenreich (TF) antigens (TFAs). The antibody of the present invention can also exhibit specificity for a cancer-associated protein. Such proteins can include any protein associated with cancer progression. Examples of such proteins include angiogenesis factors associated with tumor growth, such as vascular endothelial growth factors (VEGFs), fibroblast growth factors (FGFs), tissue factor (TF), epidermal growth factors (EGFs), and receptors thereof; factors associated with tumor invasiveness; and other receptors associated

with cancer progression (e.g., one of the HER1, HER2, HER3 or HER4 receptors). Accordingly the antibody of the present invention is specific for on antigen selected from the group consisting of 4-IBB, 5T4, adenocarcinoma antigen, alpha-fetoprotein, BAFF, B-lymphoma cell, C242 antigen, CA- 125, carbonic anhydrase 9 (CA-IX), C-MET, CCR4, CD 152, CD 19, CD20, CD200, CD22, CD221, CD23 (IgE receptor), CD28, CD30 (TNFRSF8), CD33, CD4, CD40, CD44 v6, CD51, CD52, CD56, CD74, CD80, CEA, CNT0888, CTLA-4, DR5, EGFR, EpCAM, CD3, FAP, fibronectin extra domain-B, folate receptor 1, GD2, GD3 ganglioside, glycoprotein 75, GPNMB, HER2/neu, HGF, human scatter factor receptor kinase, IGF-1 receptor, IGF-I, IgG1, LI -CAM, IL-13, IL-6, insulin- like growth factor I receptor, integrin $\alpha 5\beta 1$, integrin $\alpha v\beta 3$, MORAb-009, MS4A1, MUC1, mucin CanAg, N-glycolylneuraminic acid, NPC-1C, PDGF-R a, PDL192, phosphatidylserine, prostatic carcinoma cells, RANKL, RON, ROR1, SCH 900105, SDC1, SLAMF7, TAG-72, tenascin C, TGF beta 2, TGF- β , TRAIL-R1, TRAIL-R2, tumor antigen CTAA16.88, VEGF-A, VEGFR-1, VEGFR2 or vimentin. In some embodiments, the antibody of the present invention is specific for one antigen selected from the group consisting of alpha4beta7 integrin, B-cell activating factor, CD11a, CD19, CD20, CD3, CD30, CD33, CD52, CTLA-4 (cytotoxic T-lymphocyte-associated protein 4), EGFR, EPCAM, GD2, GPIIb/IIIa, HER2, IgE, IL2R, IL6R, PD1, RSV F protein, VEGF and VEGFR2.

In some embodiments, the antibody of the present invention is specific for one CD molecule selected from the group consisting of CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3delta, CD3epsilon, CD3gamma, CD4, CD5, CD6, CD7, CD8alpha, CD8beta, CD9, CD10, CD11a, CD11b, CD11c, CDw12, CD13, CD14, CD15u, CD16a, CD16b, CDw17, CD18, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD26, CD27, CD28, CD29, CD30, CD31, CD32, CD33, CD34, CD35, CD36, CD37, CD38, CD39, CD40, CD41, CD42a, CD42b, CD42c, CD42d, CD43, CD44, CD44R, CD45, CD46, CD47R, CD48, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD50, CD51, CD52, CD53, CD54, CD55, CD56, CD57, CD58, CD59, CD60a, CD60b, CD60c, CD61, CD62E, CD62L, CD62P, CD63, CD64, CD65, CD65s, CD66a, CD66b, CD66c, CD66d, CD66e, CD66f, CD68, CD69, CD70, CD71, CD72, CD73, CD74, CD75, CD75s, CD77, CD79a, CD79b, CD80, CD81, CD82, CD83, CD84, CD85, CD86, CD87, CD88, CD89, CD90, CD91, CD92, CDw93, CD94, CD95, CD96, CD97, CD98, CD99, CD100, CD101, CD102, CD103, CD104, CD105, CD106, CD107a, CD107b, CD108, CD109, CD110, CD111, CD112, CDw113, CD114, CD115, CD116, CD117, CD118, CDw119, CD120a, CD120b, CD121a, CDw121b, CD122, CD123,

CD124, CDw125, CD126, CD127, CDw128a, CDw128b, CD129, CD130, CD131, CD132, CD133, CD134, CD135, CDw136, CDw137, CD138, CD139, CD140a, CD140b, CD141, CD142, CD143, CD144, CDw145, CD146, CD147, CD148, CDw149, CD150, CD151, CD152, CD153, CD154, CD155, CD156a, CD156b, CDw156C, CD157, CD158, CD159a, CD159c, CD160, CD161, CD162, CD162R, CD163, CD164, CD165, CD166, CD167a, CD168, CD169, CD170, CD171, CD172a, CD172b, CD172g, CD173, CD174, CD175, CD175s, CD176, CD177, CD178, CD179a, CD179b, CD180, CD181, CD182, CD183, CD184, CD185, CDw186, CD191, CD192, CD193, CD195, CD196, CD197, CDw198, CDw199, CDw197, CD200, CD201, CD202b, CD203c, CD204, CD205, CD206, CD207, CD208, CD209, CDw210, CD212, CD213a1, CD213a2, CDw217, CDw218a, CDw218b, CD220, CD221, CD222, CD223, CD224, CD225, CD226, CD227, CD228, CD229, CD230, CD231, CD232, CD233, CD234, CD235a, CD235b, CD235ab, CD236, CD236R, CD238, CD239, CD240CE, CD240D, CD240DCE, CD241, CD242, CD243, CD244, CD245, CD246, CD247, CD248, CD249, CD252, CD253, CD254, CD256, CD257, CD258, CD261, CD262, CD263, CD264, CD265, CD266, CD267, CD268, CD269, CD271, CD272, CD273, CD274, CD275, CD276, CD277, CD278, CD279, CD280, CD281, CD282, CD283, CD284, CD289, CD292, CDw293, CD294, CD295, CD296, CD297, CD298, CD299, CD300a, CD300c, CD300e, CD301, CD302, CD303, CD304, CD305, CD306, CD307, CD309, CD312, CD314, CD315, CD316, CD317, CD318, CD319, CD320, CD321, CD322, CD324, CDw325, CD326, CDw327, CDw328, CDw329, CD331, CD332, CD333, CD334, CD335, CD336, CD337, CDw338, and CD339.

In some embodiments, the antibody of the present invention derives a monoclonal antibody selected from the group consisting of Abciximab, Adalimumab, Ado-trastuzumab emtansine, Alemtuzumab, Basiliximab, Belimumab, Bevacizumab, Blinatumomab, Brentuximab vedotin, Canakinumab, Catumaxomab, Certolizumab pegol, Cetuximab, Daclizumab, Denosumab, Dinutuximab, Eculizumab, Efalizumab, Evolocumab, Gemtuzumab ozogamicin, Golimumab, Ibritumomab tiuxetan, Infliximab, Ipilimumab, Mepolizumab, Muromonab-CD3, Natalizumab, Necitumumab, Nivolumab, Obinutuzumab, Ofatumumab, Omalizumab, Palivizumab, Panitumumab, Pembrolizumab, Pertuzumab, Ramucirumab, Ranibizumab, Raxibacumab, Rituximab, Secukinumab, Siltuximab, Tocilizumab, Tositumomab, Trastuzumab, Ustekinumab and Vedolizumab.

In some embodiments, the antibody is cetuximab. As used herein the term “cetuximab” has its general meaning in the art and refers to the antibody characterized by the heavy chain as set forth in SEQ ID NO:4 and the light chain as set forth in SEQ ID NO:5.

5 SEQ ID NO:4: Cetuximab H γ 1

QVQLKQSGPGLVQPSSQLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIW
SGGNTDYNTPFTRSLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYW
GQGTLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKT
10 HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDG
VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

15 SEQ ID NO:5: Cetuximab L kappa

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRRTNGSPRLLIKYASESIS
GIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPS
VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS
20 TYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

In another aspect, the present invention relates to the antibody of the present invention, as defined in any aspect or embodiment herein, for use as a medicament.

In another aspect, the present invention relates to a method of treating cancer in a
25 subject in need thereof comprising administering the subject with a therapeutically effective amount of an antibody of the present invention.

As used herein, “treatment” or “treating” is an approach for obtaining beneficial or desired results including clinical results. For purposes of this invention, beneficial or desired
30 clinical results include, but are not limited to, one or more of the following: alleviating one or more symptoms resulting from the disease, diminishing the extent of the disease, stabilizing the disease (e.g., preventing or delaying the worsening of the disease), preventing or delaying the spread (e.g., metastasis) of the disease, preventing or delaying the recurrence of the disease, delay or slowing the progression of the disease, ameliorating the disease state,

providing a remission (partial or total) of the disease, decreasing the dose of one or more other medications required to treat the disease, delaying the progression of the disease, increasing the quality of life, and/or prolonging survival. Also encompassed by "treatment" is a reduction of pathological consequence of cancer. The methods of the present invention
5 contemplate any one or more of these aspects of treatment.

In particular, the antibody of the invention is particularly suitable for promoting apoptosis of cancer cells that express OX1R. The antibody of the present invention can thus combine 2 functions: one that is brought by with the antigen binding site, namely specificity
10 for one antigen and the other one that is brought by the orexin polypeptide, namely promotion of apoptosis. Accordingly the antibody of the present invention is thus both capable of targeting a cancer cell by binding to the particular antigen and promoting apoptosis. The binding of the antigen can also be associated with a therapeutic effect (i.e. inhibition of the receptor).

As used herein, the term "cancer" has its general meaning in the art and includes, but is not limited to, solid tumors and blood borne tumors. The term cancer includes diseases of the skin, tissues, organs, bone, cartilage, blood and vessels. The term "cancer" further encompasses both primary and metastatic cancers. Examples of cancers that may treated by
20 methods and compositions of the invention include, but are not limited to, cancer cells from the bladder, blood, bone, bone marrow, brain, breast, colon, esophagus, gastrointestinal, gum, head, kidney, liver, lung, nasopharynx, neck, ovary, prostate, skin, stomach, testis, tongue, or uterus. In addition, the cancer may specifically be of the following histological type, though it is not limited to these: neoplasm, malignant; carcinoma; carcinoma, undifferentiated; giant
25 and spindle cell carcinoma; small cell carcinoma; papillary carcinoma; squamous cell carcinoma; lymphoepithelial carcinoma; basal cell carcinoma; pilomatrix carcinoma; transitional cell carcinoma; papillary transitional cell carcinoma; adenocarcinoma; gastrinoma, malignant; cholangiocarcinoma; hepatocellular carcinoma; combined hepatocellular carcinoma and cholangiocarcinoma; trabecular adenocarcinoma; adenoid cystic
30 carcinoma; adenocarcinoma in adenomatous polyp; adenocarcinoma, familial polyposis coli; solid carcinoma; carcinoid tumor, malignant; branchiolo-alveolar adenocarcinoma; papillary adenocarcinoma; chromophobe carcinoma; acidophil carcinoma; oxyphilic adenocarcinoma; basophil carcinoma; clear cell adenocarcinoma; granular cell carcinoma; follicular adenocarcinoma; papillary and follicular adenocarcinoma; nonencapsulating sclerosing

carcinoma; adrenal cortical carcinoma; endometroid carcinoma; skin appendage carcinoma; apocrine adenocarcinoma; sebaceous adenocarcinoma; ceruminous; adenocarcinoma; mucoepidermoid carcinoma; cystadenocarcinoma; papillary cystadenocarcinoma; papillary serous cystadenocarcinoma; mucinous cystadenocarcinoma; mucinous adenocarcinoma; 5 signet ring cell carcinoma; infiltrating duct carcinoma; medullary carcinoma; lobular carcinoma; inflammatory carcinoma; paget's disease, mammary; acinar cell carcinoma; adenosquamous carcinoma; adenocarcinoma w/squamous metaplasia; thymoma, malignant; ovarian stromal tumor, malignant; thecoma, malignant; granulosa cell tumor, malignant; and roblastoma, malignant; Sertoli cell carcinoma; leydig cell tumor, malignant; lipid cell tumor, 10 malignant; paraganglioma, malignant; extra-mammary paraganglioma, malignant; pheochromocytoma; glomangiosarcoma; malignant melanoma; amelanotic melanoma; superficial spreading melanoma; malig melanoma in giant pigmented nevus; epithelioid cell melanoma; blue nevus, malignant; sarcoma; fibrosarcoma; fibrous histiocytoma, malignant; myxosarcoma; liposarcoma; leiomyosarcoma; rhabdomyosarcoma; embryonal 15 rhabdomyosarcoma; alveolar rhabdomyosarcoma; stromal sarcoma; mixed tumor, malignant; mullerian mixed tumor; nephroblastoma; hepatoblastoma; carcinosarcoma; mesenchymoma, malignant; brenner tumor, malignant; phyllodes tumor, malignant; synovial sarcoma; mesothelioma, malignant; dysgerminoma; embryonal carcinoma; teratoma, malignant; struma ovarii, malignant; choriocarcinoma; mesonephroma, malignant; hemangiosarcoma; 20 hemangioendothelioma, malignant; kaposi's sarcoma; hemangiopericytoma, malignant; lymphangiosarcoma; osteosarcoma; juxtacortical osteosarcoma; chondrosarcoma; chondroblastoma, malignant; mesenchymal chondrosarcoma; giant cell tumor of bone; ewing's sarcoma; odontogenic tumor, malignant; ameloblastic odontosarcoma; ameloblastoma, malignant; ameloblastic fibrosarcoma; pinealoma, malignant; chordoma; 25 glioma, malignant; ependymoma; astrocytoma; protoplasmic astrocytoma; fibrillary astrocytoma; astroblastoma; glioblastoma; oligodendroglioma; oligodendroblastoma; primitive neuroectodermal; cerebellar sarcoma; ganglioneuroblastoma; neuroblastoma; retinoblastoma; olfactory neurogenic tumor; meningioma, malignant; neurofibrosarcoma; neurilemmoma, malignant; granular cell tumor, malignant; malignant lymphoma; Hodgkin's 30 disease; Hodgkin's lymphoma; paraganuloma; malignant lymphoma, small lymphocytic; malignant lymphoma, large cell, diffuse; malignant lymphoma, follicular; mycosis fungoides; other specified non-Hodgkin's lymphomas; malignant histiocytosis; multiple myeloma; mast cell sarcoma; immunoproliferative small intestinal disease; leukemia; lymphoid leukemia; plasma cell leukemia; erythroleukemia; lymphosarcoma cell leukemia; myeloid leukemia;

basophilic leukemia; eosinophilic leukemia; monocytic leukemia; mast cell leukemia; megakaryoblastic leukemia; myeloid sarcoma; and hairy cell leukemia.

In some embodiments, the subject suffers from an epithelial cancer. As used herein, the term “epithelial cancer” refers to any malignant process that has an epithelial origin. Examples of epithelial cancers include, but are not limited to, a gynecological cancer such as endometrial cancer, ovarian cancer, cervical cancer, vulvar cancer, uterine cancer or fallopian tube cancer, breast cancer, prostate cancer, lung cancer, pancreatic cancer, urinary cancer, bladder cancer, head and neck cancer, oral cancer colorectal cancer and liver cancer. An epithelial cancer may be at different stages as well as varying degrees of grading. In some embodiments, the epithelial cancer is selected from the group consisting of breast cancer, prostate cancer, lung cancer, pancreatic cancer, bladder cancer, colorectal cancer and ovarian cancer. In some embodiments, the epithelial cancer is a colorectal cancer. In some embodiments, the epithelial cancer is a liver cancer, in particular a hepatocellular carcinoma. In some embodiments, the epithelial cancer is breast cancer. In some embodiments, the epithelial cancer is ovarian cancer. In some embodiments, the epithelial cancer is prostate cancer, in particular advanced prostate cancer. In some embodiments, the epithelial cancer is lung cancer. In some embodiments, the epithelial cancer is head and neck cancer. In some embodiments, the epithelial cancer is head and neck squamous cell carcinoma. As used herein the term “pancreatic cancer” or “pancreas cancer” as used herein 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). As used herein the term “hepatocellular carcinoma” has its general meaning in the art and refers to the cancer developed in hepatocytes. In general, liver cancer indicates hepatocellular carcinoma in large. HCC may be caused by an infectious agent such as hepatitis B virus (HBV, hereinafter may be referred to as HBV) or hepatitis C virus (HCV, hereinafter may be referred to as HCV). In some embodiments, HCC results from alcoholic steatohepatitis or non-alcoholic steatohepatitis (hereinafter may be abbreviated to as “NASH”). In some embodiments, the HCC is early stage HCC, non-metastatic HCC, primary HCC, advanced HCC, locally advanced HCC, metastatic HCC, HCC in remission, or recurrent HCC. In some embodiments, the HCC is localized resectable (i.e., tumors that are confined to a portion of the liver that allows for complete surgical removal), localized unresectable (i.e., the localized

tumors may be unresectable because crucial blood vessel structures are involved or because the liver is impaired), or unresectable (i.e., the tumors involve all lobes of the liver and/or has spread to involve other organs (e.g., lung, lymph nodes, bone). In some embodiments, the HCC is, according to TNM classifications, a stage I tumor (single tumor without vascular invasion), a stage II tumor (single tumor with vascular invasion, or multiple tumors, none greater than 5 cm), a stage III tumor (multiple tumors, any greater than 5 cm, or tumors involving major branch of portal or hepatic veins), a stage IV tumor (tumors with direct invasion of adjacent organs other than the gallbladder, or perforation of visceral peritoneum), N1 tumor (regional lymph node metastasis), or M1 tumor (distant metastasis). In some embodiments, the HCC is, according to AJCC (American Joint Commission on Cancer) staging criteria, stage T1, T2, T3, or T4 HCC. As used herein the term "advanced prostate cancer" has its general meaning in the art. "Castration resistant prostate cancer," "CaP," "androgen-receptor dependent prostate cancer," "androgen-independent prostate cancer," are used interchangeably to refer to prostate cancer in which prostate cancer cells "grow" (i.e., increase in number) in the absence of androgens and/or in the absence of expression of androgen receptors on the cancer cells.

In some embodiments, the antibody of the present invention is particularly suitable for the treatment of metastatic colorectal cancer, in particular metastatic colorectal cancer associated with at least one RAS mutation, in particular at least one KRAS mutation. The term "RAS mutation" has its general meaning in the art and refers to the mutations in the Ras family of proto-oncogenes (comprising H-Ras, N-Ras, K-Ras, DIRAS1; DIRAS2; DIRAS3; ERAS; GEM; MRAS; NKIRAS1; NKIRAS2; NRAS; RALA; RALB; RAP1A; RAP1B; RAP2A; RAP2B; RAP2C; RASD1; RASD2; RASL10A; RASL10B; RASL11A; RASL11B; RASL12; REM1; REM2; RERG; RERGL; RRAD; RRAS; RRAS2). In particular, the term "KRAS mutation" includes any one or more mutations in the KRAS (which can also be referred to as KRAS2 or RASK2) gene. For example, the KRAS mutations are located in exon 3 or exon 4 of the gene. Examples of KRAS mutations include, but are not limited to, G12C, G12D, G13D, G12R, G12S, and G12V. KRAS is one of the commonly mutated oncogenes in human cancers. In particular, KRAS mutations are found in 30-40% of tumors and represent together with APC one of the somatic alteration involved in the initiation of colorectal cancer. This mutation occurs early in the process of carcinogenesis, and is maintained at the various stages of disease progression, such as node involvement and metastatic spread. A recent study involving a large number of patients has demonstrated that

mutated KRAS is associated with worse outcome in colorectal cancer progression, with effects being more pronounced in stage II and III disease (Nash, et al., Ann. Surg. Oncol., 17: 416- 424, 2010). The same group has shown, in another study (Nash, et al., Ann. Surg. Oncol., 17: 572-578, 2010), that KRAS mutation is associated with more rapid and aggressive metastatic behavior of colorectal liver metastases. In addition, KRAS mutation has been reported to induce drug resistance and treatment failure to epidermal-growth factor receptor (EGFR)-targeting therapeutics in metastatic colorectal cancer. KRAS mutations confer resistance to both cetuximab (Erbix®) and panitumumab (Vectibix®) (Allegra et al., J. Clin. Oncol., 27: 2091 -2096, 2008; Linardou et al., Lancet Oncol., 9: 962-972, 2008).

In some embodiments, the antibody of the present invention is particularly suitable for the treatment of metastatic colorectal cancer, in particular metastatic colorectal cancer associated with at least one BRAF mutation. The term "BRAF mutation" includes any one or more mutations in the BRAF (which can also be referred to as serine/threonine -protein kinase B-Raf or B-Raf) gene. Typically, the BRAF mutation is V600E. The serine-threonine kinase BRAF is the principal effector of KRAS and BRAF wild-type had been shown to be required for response to panitumumab or cetuximab and is used to select patients who are eligible for the treatment.

In some embodiments, the present invention relates to a method of treating a metastatic colorectal cancer associated with a RAS (e.g. KRAS) or BRAF mutation in a subject in need thereof comprising administering a therapeutically effective amount of an antibody of the present invention wherein said antibody derives from cetuximab.

As used herein, the term "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. A therapeutically effective amount of an antibody of the present invention may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody of the present invention to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects. The efficient dosages and dosage regimens for the antibody of the present invention depend on the disease or condition to be treated and may be determined by the persons skilled in the art. A physician having ordinary skill in the art may readily determine and prescribe the effective

amount of the pharmaceutical composition required. For example, the physician could start doses of the antibody of the present invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. In general, a suitable dose of a composition of the present invention will be that amount of the compound which is the lowest dose effective to produce a therapeutic effect according to a particular dosage regimen. Such an effective dose will generally depend upon the factors described above. For example, a therapeutically effective amount for therapeutic use may be measured by its ability to stabilize the progression of disease. The ability of a compound to inhibit cancer may, for example, be evaluated in an animal model system predictive of efficacy in human tumors. Alternatively, this property of a composition may be evaluated by examining the ability of the compound to inhibit cell growth or to induce cytotoxicity by in vitro assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound may decrease tumor size, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected. An exemplary, non-limiting range for a therapeutically effective amount of an antibody of the present invention is about 0.1-100 mg/kg, such as about 0.1-50 mg/kg, for example about 0.1-20 mg/kg, such as about 0.1-10 mg/kg, for instance about 0.5, about such as 0.3, about 1, about 3 mg/kg, about 5 mg/kg or about 8 mg/kg. An exemplary, non-limiting range for a therapeutically effective amount of an antibody of the present invention is 0.02-100 mg/kg, such as about 0.02-30 mg/kg, such as about 0.05-10 mg/kg or 0.1-3 mg/kg, for example about 0.5-2 mg/kg. Administration may e.g. be intravenous, intramuscular, intraperitoneal, or subcutaneous, and for instance administered proximal to the site of the target. Dosage regimens in the above methods of treatment and uses are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. In some embodiments, the efficacy of the treatment is monitored during the therapy, e.g. at predefined points in time. In some embodiments, the efficacy may be monitored by measuring both the levels of OX1R and the cancer antigen for which the antibody is specific in a sample containing tumor cells, by visualization of the disease area, or by other diagnostic methods described further herein, e.g. by performing one or more PET-CT scans, for example using a labeled human monoclonal antibody of the present invention, fragment or mini-antibody

derived from the antibody of the present invention. If desired, an effective daily dose of a pharmaceutical composition may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms. In some embodiments, antibody of the present invention are administered by
5 slow continuous infusion over a long period, such as more than 24 hours, in order to minimize any unwanted side effects. An effective dose of an antibody of the present invention may also be administered using a weekly, biweekly or triweekly dosing period. The dosing period may be restricted to, e.g., 8 weeks, 12 weeks or until clinical progression has been established. As non-limiting examples, treatment according to the present invention may be provided as a
10 daily dosage of a compound of the present invention in an amount of about 0.1-100 mg/kg, such as 0.2, 0.5, 0.9, 1.0, 1.1, 1.5, 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, 30, 40, 45, 50, 60, 70, 80, 90 or 100 mg/kg, per day, on at least one of days 1, 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, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, or alternatively, at least one
15 of weeks 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 after initiation of treatment, or any combination thereof, using single or divided doses every 24, 12, 8, 6, 4, or 2 hours, or any combination thereof.

The present invention also provides for therapeutic applications where an antibody of
20 the present invention is used in combination with at least one further therapeutic agent for treating cancer. Such administration may be simultaneous, separate or sequential. For simultaneous administration the agents may be administered as one composition or as separate compositions, as appropriate.

25 The further therapeutic agent is typically relevant for the disorder to be treated. Exemplary therapeutic agents include other anti-cancer antibodies, cytotoxic agents, chemotherapeutic agents, anti-angiogenic agents, anti-cancer immunogens, cell cycle control/apoptosis regulating agents, hormonal regulating agents, and other agents described below.

30 In one aspect, the further therapeutic agent is at least one second antibody which binds another target such as, e.g., CC1, CD5, CD8, CD14, CD15, CD19, CD21, CD22, CD23, CD25, CD30, CD33, CD37, CD38, CC10, CC10L, CC16, CD52, CD54, CD80, CD126, B7, MUC1, tenascin, HM 1.24, or HLA-DR. For example, the second antibody may bind to a B

cell antigen, including, but not limited to CD20, CD19, CD21, CD23, CD38, CC16, CD80, CD138, HLA-DR, CD22, or to another epitope on OX1R. In some embodiments, the second antibody binds vascular endothelial growth factor A (VEGF-A). In some embodiments, the antibody of the present invention is for use in combination with a specific therapeutic antibody. Monoclonal antibodies currently used as cancer immunotherapeutic agents that are suitable for inclusion in the combinations of the present invention include, but are not limited to, rituximab (Rituxan®), trastuzumab (Herceptin®), ibritumomab tiuxetan (Zevalin®), tositumomab (Bexxar®), cetuximab (C-225, Erbitux®), bevacizumab (Avastin®), gemtuzumab ozogamicin (Mylotarg®), alemtuzumab (Campath®), and BL22. Other examples include anti-CTLA4 antibodies (e.g. Ipilimumab), anti-PD1 antibodies, anti-PDL1 antibodies, anti-TIMP3 antibodies, anti-LAG3 antibodies, anti-B7H3 antibodies, anti-B7H4 antibodies or anti-B7H6 antibodies. In some embodiments, antibodies include B cell depleting antibodies. Typical B cell depleting antibodies include but are not limited to anti-CD20 monoclonal antibodies [e.g. Rituximab (Roche), Ibritumomab tiuxetan (Bayer Schering), Tositumomab (GlaxoSmithKline), AME-133v (Applied Molecular Evolution), Ocrelizumab (Roche), Ofatumumab (HuMax-CD20, Gemnab), TRU-015 (Trubion) and IMMU-106 (Immunomedics)], an anti-CD22 antibody [e.g. Epratuzumab, Leonard et al., Clinical Cancer Research (Z004) 10: 53Z7-5334], anti-CD79a antibodies, anti-CD27 antibodies, or anti-CD19 antibodies (e.g. U.S. Pat. No. 7,109,304), anti-BAFF-R antibodies (e.g. Belimumab, GlaxoSmithKline), anti-APRIL antibodies (e.g. anti-human APRIL antibody, ProSci inc.), and anti-IL-6 antibodies [e.g. previously described by De Benedetti et al., J Immunol (2001) 166: 4334-4340 and by Suzuki et al., Europ J of Immunol (1992) 22 (8) 1989-1993, fully incorporated herein by reference].

In some embodiments, the antibody of the present invention is used in combination with a chemotherapeutic agent. The term "chemotherapeutic agent" refers to chemical compounds that are effective in inhibiting tumor growth. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatins; callistatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly

cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CBI-TMI); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estrarnustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, 5 melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as the enediyne antibiotics (e.g. calicheamicin, especially calicheamicin (11 and calicheamicin 211, see, e.g., Agnew Chem Intl. Ed. Engl. 33:183-186 (1994); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin 10 chromophore and related chromoprotein enediyne antiobiotic chromomorphores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabycin, canninomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, 15 esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalarnycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptomycin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, 20 thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitestosterone, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic 25 acid; amsacrine; bestabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®; razoxane; rhizoxin; sizofiran; spirogennanum; 30 tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethane; vindesine; dacarbazine; mannomustine; mitobromtol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g. paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.J.) and doxorubicin (TAXOTERE®, Rhone-Poulenc Rorer, Antony, France); chlorambucil;

gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11 ; topoisomerase inhibitor RFS 2000; 5 difluoromethylornithine (DMFO); retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are antihormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene 10 (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

In some embodiments, the antibody of the present invention is used in combination with a targeted cancer therapy. Targeted cancer therapies are drugs or other substances that 15 block the growth and spread of cancer by interfering with specific molecules ("molecular targets") that are involved in the growth, progression, and spread of cancer. Targeted cancer therapies are sometimes called "molecularly targeted drugs," "molecularly targeted therapies," "precision medicines," or similar names. In some embodiments, the targeted therapy consists of administering the subject with a tyrosine kinase inhibitor. The term "tyrosine kinase 20 inhibitor" refers to any of a variety of therapeutic agents or drugs that act as selective or non-selective inhibitors of receptor and/or non-receptor tyrosine kinases. Tyrosine kinase inhibitors and related compounds are well known in the art and described in U.S Patent Publication 2007/0254295, which is incorporated by reference herein in its entirety. It will be appreciated by one of skill in the art that a compound related to a tyrosine kinase inhibitor 25 will recapitulate the effect of the tyrosine kinase inhibitor, e.g., the related compound will act on a different member of the tyrosine kinase signaling pathway to produce the same effect as would a tyrosine kinase inhibitor of that tyrosine kinase. Examples of tyrosine kinase inhibitors and related compounds suitable for use in methods of embodiments of the present invention include, but are not limited to, dasatinib (BMS-354825), PP2, BEZ235, saracatinib, 30 gefitinib (Iressa), sunitinib (Sutent; SU11248), erlotinib (Tarceva; OSI-1774), lapatinib (GW572016; GW2016), canertinib (CI 1033), semaxinib (SU5416), vatalanib (PTK787/ZK222584), sorafenib (BAY 43-9006), imatinib (Gleevec; STI571), leflunomide (SU101), vandetanib (Zactima; ZD6474), MK-2206 (8-[4-aminocyclobutyl]phenyl]-9-phenyl-1,2,4-triazolo[3,4-f][1,6]naphthyridin-3(2H)-one hydrochloride) derivatives thereof, analogs

thereof, and combinations thereof. Additional tyrosine kinase inhibitors and related compounds suitable for use in the present invention are described in, for example, U.S. Patent Publication 2007/0254295, U.S. Pat. Nos. 5,618,829, 5,639,757, 5,728,868, 5,804,396, 6,100,254, 6,127,374, 6,245,759, 6,306,874, 6,313,138, 6,316,444, 6,329,380, 6,344,459, 6,420,382, 6,479,512, 6,498,165, 6,544,988, 6,562,818, 6,586,423, 6,586,424, 6,740,665, 6,794,393, 6,875,767, 6,927,293, and 6,958,340, all of which are incorporated by reference herein in their entirety. In some embodiments, the tyrosine kinase inhibitor is a small molecule kinase inhibitor that has been orally administered and that has been the subject of at least one Phase I clinical trial, more preferably at least one Phase II clinical, even more preferably at least one Phase III clinical trial, and most preferably approved by the FDA for at least one hematological or oncological indication. Examples of such inhibitors include, but are not limited to, Gefitinib, Erlotinib, Lapatinib, Canertinib, BMS-599626 (AC-480), Neratinib, KRN-633, CEP-11981, Imatinib, Nilotinib, Dasatinib, AZM-475271, CP-724714, TAK-165, Sunitinib, Vatalanib, CP-547632, Vandetanib, Bosutinib, Lestaurtinib, Tandutinib, Midostaurin, Enzastaurin, AEE-788, Pazopanib, Axitinib, Motasenib, OSI-930, Cediranib, KRN-951, Dovitinib, Seliciclib, SNS-032, PD-0332991, MKC-I (Ro-317453; R-440), Sorafenib, ABT-869, Brivanib (BMS-582664), SU-14813, Telatinib, SU-6668, (TSU-68), L-21649, MLN-8054, AEW-541, and PD-0325901.

In some embodiments, the antibody of the present invention is used in combination with a HER inhibitor. In some embodiments, the HER inhibitor is an EGFR inhibitor. GFR inhibitors are well known in the art (Inhibitors of erbB-1 kinase; Expert Opinion on Therapeutic Patents Dec 2002, Vol. 12, No. 12, Pages 1903-1907, Susan E Kane. Cancer therapies targeted to the epidermal growth factor receptor and its family members. Expert Opinion on Therapeutic Patents Feb 2006, Vol. 16, No. 2, Pages 147-164. Peter TrOX1Rer Tyrosine kinase inhibitors in cancer treatment (Part II). Expert Opinion on Therapeutic Patents Dec 1998, Vol. 8, No. 12, Pages 1599-1625). Examples of such agents include antibodies and small organic molecules that bind to EGFR. Examples of antibodies which bind to EGFR include MAb 579 (ATCC CRL HB 8506), MAb 455 (ATCC CRL HB8507), MAb 225 (ATCC CRL 8508), MAb 528 (ATCC CRL 8509) (see, U.S. Pat. No. 4,943,533, Mendelsohn et al.) and variants thereof, such as chimerized 225 (C225 or Cetuximab; ERBUTIX®) and reshaped human 225 (H225) (see, WO 96/40210, Imclone Systems Inc.); IMC-11F8, a fully human, EGFR-targeted antibody (Imclone); antibodies that bind type II mutant EGFR (U.S. Pat. No. 5,212,290); humanized and chimeric antibodies that bind EGFR

as described in U.S. Pat. No. 5,891,996; and human antibodies that bind EGFR, such as ABX-EGF (see WO98/50433, Abgenix); EMD 55900 (Stragliotto et al. Eur. J. Cancer 32A:636-640 (1996)); EMD7200 (matuzumab) a humanized EGFR antibody directed against EGFR that competes with both EGF and TGF- α for EGFR binding; and mAb 806 or humanized mAb 806 (Johns et al., J. Biol. Chem. 279(29):30375-30384 (2004)). The anti-EGFR antibody may be conjugated with a cytotoxic agent, thus generating an immunoconjugate (see, e.g., EP659,439A2, Merck Patent GmbH). Examples of small organic molecules that bind to EGFR include ZD1839 or Gefitinib (IRESSATM; Astra Zeneca); CP-358774 or erlotinib (TARCEVATM; Genentech/OSI); and AG1478, AG1571 (SU 5271; Sugen); EMD-7200. In some embodiments, the HER inhibitor is a small organic molecule pan-HER inhibitor such as dacomitinib (PF-00299804). In some embodiments, the HER inhibitor is selected from the group consisting of cetuximab, panitumumab, zalutumumab, nimotuzumab, erlotinib, gefitinib, lapatinib, neratinib, canertinib, vandetanib, afatinib, TAK-285 (dual HER2 and EGFR inhibitor), ARRY334543 (dual HER2 and EGFR inhibitor), Dacomitinib (pan-ErbB inhibitor), OSI-420 (Desmethyl Erlotinib) (EGFR inhibitor), AZD8931 (EGFR, HER2 and HER3 inhibitor), AEE788 (NVP-AEE788) (EGFR, HER2 and VEGFR 1/2 inhibitor), Pelitinib (EKB-569) (pan-ErbB inhibitor), CUDC-101 (EGFR, HER2 and HDAC inhibitor), XL647 (dual HER2 and EGFR inhibitor), BMS-599626 (AC480) (dual HER2 and EGFR inhibitor), PKC412 (EGFR, PKC, cyclic AMP-dependent protein kinase and S6 kinase inhibitor), BIBX1382 (EGFR inhibitor) and AP261 13 (ALK and EGFR inhibitor). The inhibitors cetuximab, panitumumab, zalutumumab, nimotuzumab are monoclonal antibodies. erlotinib, gefitinib, lapatinib, neratinib, canertinib, vandetanib and afatinib are tyrosine kinase inhibitors.

In some embodiments, the antibody of the present invention is used in combination with a c-Met inhibitor. In some embodiments the c-Met inhibitor is an anti-c-Met antibody. In some embodiments, the anti-c-met antibody is MetMab (onartuzumab) or a biosimilar version thereof. MetMab is disclosed in, for example, WO2006/015371; Jin et al, Cancer Res (2008) 68:4360. Other anti-c-met antibodies suitable for use in the methods of the present invention are described herein and known in the art. For example, anti-c-met antibodies disclosed in WO05/016382 (including but not limited to antibodies 13.3.2, 9.1.2, 8.70.2, 8.90.3); an anti-c-met antibodies produced by the hybridoma cell line deposited with ICLC number PD 03001 at the CBA in Genoa, or that recognizes an epitope on the extracellular domain of the β chain of the HGF receptor, and said epitope is the same as that recognized by

the monoclonal antibody); anti-c- met antibodies disclosed in WO2007/126799 (including but not limited to 04536, 05087, 05088, 05091, 05092, 04687, 05097, 05098, 05100, 05101, 04541, 05093, 05094, 04537, 05102, 05105, 04696, 04682); anti c-met antibodies disclosed in WO2009/007427 (including but not limited to an antibody deposited at CNCM, Institut
5 Pasteur, Paris, France, on March 14, 2007 under the number 1-3731, on March 14, 2007 under the number 1-3732, on July 6, 2007 under the number 1-3786, on March 14, 2007 under the number 1-3724; an anti-c-met antibody disclosed in 20110129481; an anti-c-met antibody disclosed in US20110104176; an anti-c-met antibody disclosed in WO2009/134776; an anti-c-met antibody disclosed in WO2010/059654; an anti-c-met antibody disclosed in
10 WO2011020925 (including but not limited to an antibody secreted from a hybridoma deposited at the CNCM, Institut Pasteur, Paris, France, on march 12, 2008 under the number 1-3949 and the hybridoma deposited on January 14, 2010 under the number 1-4273). In some embodiments, the cMET inhibitor is selected from the group consisting of K-252a; SU-11274; PHA-665752 and other cMET inhibitors described in WO 2002/096361; AM7; AMG-
15 208 and other cMet inhibitors described in WO 2009/091374; JNJ-38877605 and other cMet inhibitors described in WO 2007/075567; MK-2461 and other cMet inhibitors described in WO 2007/002254 and/or WO 2007/002258; PF-04217903 and other cMet inhibitors described in WO 2007/132308; BMS 777607; GSK 136089 (also known as XL-880 and Foretinib) and other cMET inhibitors described in WO 2005/030140; BMS 907351 (also
20 known as XL-184); EMD 1214063; LY 2801653; ARQ 197; MK 8033; PF 2341066 and other cMET inhibitors described in WO 2006/021881; MGCD 265; E 7050; MP 470; SGX 523; cMet inhibitors described in Kirin J.J. Cui, Inhibitors targeting hepatocyte growth factor receptor and their potential therapeutic applications. Expert Opin. Ther. Patents 2007; 17: 1035-45; cMet inhibitors described in WO 2008/103277; cMet inhibitors described in WO
25 2008/008310; cMet inhibitors described in WO 2007/138472; cMet inhibitors described in WO 2008/008539; cMet inhibitors described in WO 2009/007390; cMet inhibitors described in WO 2009/053737; cMet inhibitors described in WO 2009/024825; cMet inhibitors described in WO 2008/071451; cMet inhibitors described in WO 2007/130468; cMet inhibitors described in WO 2008/051547; cMet inhibitors described in WO 2008/053157;
30 cMet inhibitors described in WO 2008/017361; WO 2008/145242; WO2008/145243; WO 2008/148449; WO 2009/007074; WO 2009/006959; WO 2009/024221; WO 2009/030333; and/or WO 2009/083076; cMet inhibitors described in WO 2009/093049; cMet inhibitors described in US 2008/039457; cMet inhibitors described in WO 2007/149427; cMet inhibitors described in WO 2007/050309; cMet inhibitors described in WO 2009/056692;

cMet inhibitors described in WO 2009/087305; cMet inhibitors described in US 2009/197864; cMet inhibitors described in US 2009/197862; cMet inhibitors described in US 2009/156594; cMet inhibitors described in WO 2008/124849; cMet inhibitors described in WO 2008/067119; cMet inhibitors described in WO 2007/064797; cMet inhibitors described in WO 2009/045992; cMet inhibitors described in WO 2008/088881; cMet inhibitors described in WO 2007/081978; cMet inhibitors described in WO 2008/079294; cMet inhibitors described in WO 2008/079291; cMet inhibitors described in WO 2008/086014; cMet inhibitors described in WO 2009/033084; cMet inhibitors described in WO 2007/059202; cMet inhibitors described in US 2009/170896; cMet inhibitors described in WO 2009/077874 and/or WO 2007/023768; cMet inhibitors described in WO 2008/049855; cMet inhibitors described in WO 2009/026717; and cMet inhibitors described in WO 2008/046216.

In some embodiments, the antibody of the present invention is used in combination with an immunotherapeutic agent. The term "immunotherapeutic agent," as used herein, refers to a compound, composition or treatment that indirectly or directly enhances, stimulates or increases the body's immune response against cancer cells and/or that decreases the side effects of other anticancer therapies. Immunotherapy is thus a therapy that directly or indirectly stimulates or enhances the immune system's responses to cancer cells and/or lessens the side effects that may have been caused by other anti-cancer agents. Immunotherapy is also referred to in the art as immunologic therapy, biological therapy biological response modifier therapy and biotherapy. Examples of common immunotherapeutic agents known in the art include, but are not limited to, cytokines, cancer vaccines, monoclonal antibodies and non-cytokine adjuvants. Alternatively the immunotherapeutic treatment may consist of administering the subject with an amount of immune cells (T cells, NK, cells, dendritic cells, B cells...).

Immunotherapeutic agents can be non-specific, i.e. boost the immune system generally so that the human body becomes more effective in fighting the growth and/or spread of cancer cells, or they can be specific, i.e. targeted to the cancer cells themselves immunotherapy regimens may combine the use of non-specific and specific immunotherapeutic agents.

Non-specific immunotherapeutic agents are substances that stimulate or indirectly improve the immune system. Non-specific immunotherapeutic agents have been used alone as

a main therapy for the treatment of cancer, as well as in addition to a main therapy, in which case the non-specific immunotherapeutic agent functions as an adjuvant to enhance the effectiveness of other therapies (e.g. cancer vaccines). Non-specific immunotherapeutic agents can also function in this latter context to reduce the side effects of other therapies, for example, bone marrow suppression induced by certain chemotherapeutic agents. Non-specific immunotherapeutic agents can act on key immune system cells and cause secondary responses, such as increased production of cytokines and immunoglobulins. Alternatively, the agents can themselves comprise cytokines. Non-specific immunotherapeutic agents are generally classified as cytokines or non-cytokine adjuvants.

A number of cytokines have found application in the treatment of cancer either as general non-specific immunotherapies designed to boost the immune system, or as adjuvants provided with other therapies. Suitable cytokines include, but are not limited to, interferons, interleukins and colony-stimulating factors. Interferons (IFNs) contemplated by the present invention include the common types of IFNs, IFN-alpha (IFN- α), IFN-beta (IFN- β) and IFN-gamma (IFN- γ). IFNs can act directly on cancer cells, for example, by slowing their growth, promoting their development into cells with more normal behaviour and/or increasing their production of antigens thus making the cancer cells easier for the immune system to recognise and destroy. IFNs can also act indirectly on cancer cells, for example, by slowing down angiogenesis, boosting the immune system and/or stimulating natural killer (NK) cells, T cells and macrophages. Recombinant IFN-alpha is available commercially as Roferon (Roche Pharmaceuticals) and Intron A (Schering Corporation). Interleukins contemplated by the present invention include IL-2, IL-4, IL-11 and IL-12. Examples of commercially available recombinant interleukins include Proleukin® (IL-2; Chiron Corporation) and Neumega® (IL-12; Wyeth Pharmaceuticals). Zymogenetics, Inc. (Seattle, Wash.) is currently testing a recombinant form of IL-21, which is also contemplated for use in the combinations of the present invention. Colony-stimulating factors (CSFs) contemplated by the present invention include granulocyte colony stimulating factor (G-CSF or filgrastim), granulocyte-macrophage colony stimulating factor (GM-CSF or sargramostim) and erythropoietin (epoetin alfa, darbepoietin). Treatment with one or more growth factors can help to stimulate the generation of new blood cells in subjects undergoing traditional chemotherapy. Accordingly, treatment with CSFs can be helpful in decreasing the side effects associated with chemotherapy and can allow for higher doses of chemotherapeutic agents to be used. Various-recombinant colony stimulating factors are available commercially, for example, Neupogen® (G-CSF; Amgen),

Neulasta (pelfilgrastim; Amgen), Leukine (GM-CSF; Berlex), Procrit (erythropoietin; Ortho Biotech), Epogen (erythropoietin; Amgen), Arnesp (erythropoietin).

Combination compositions and combination administration methods of the present invention may also involve "whole cell" and "adoptive" immunotherapy methods. For instance, such methods may comprise infusion or re-infusion of immune system cells (for instance tumor-infiltrating lymphocytes (TILs), such as CC1+ and/or CD8+ T cells (for instance T cells expanded with tumor-specific antigens and/or genetic enhancements), antibody-expressing B cells or other antibody-producing or -presenting cells, dendritic cells (e.g., dendritic cells cultured with a DC-expanding agent such as GM-CSF and/or Flt3-L, and/or tumor-associated antigen-loaded dendritic cells), anti-tumor NK cells, so-called hybrid cells, or combinations thereof. Cell lysates may also be useful in such methods and compositions. Cellular "vaccines" in clinical trials that may be useful in such aspects include Canvaxin™, APC-8015 (Dendreon), HSPPC-96 (Antigenics), and Melacine® cell lysates. Antigens shed from cancer cells, and mixtures thereof (see for instance Bystryn et al., Clinical Cancer Research Vol. 7, 1882-1887, July 2001), optionally admixed with adjuvants such as alum, may also be components in such methods and combination compositions.

In some embodiments, the antibody of the present invention is used in combination with radiotherapy. Radiotherapy may comprise radiation or associated administration of radiopharmaceuticals to a patient. The source of radiation may be either external or internal to the patient being treated (radiation treatment may, for example, be in the form of external beam radiation therapy (EBRT) or brachytherapy (BT)). Radioactive elements that may be used in practicing such methods include, e.g., radium, cesium-137, iridium-192, americium-241, gold-198, cobalt-57, copper-67, technetium-99, iodide-123, iodide-131, and indium-111.

For administration, the antibody of the present invention is formulated as a pharmaceutical composition. A pharmaceutical composition comprising an antibody of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the therapeutic molecule is combined in a mixture with a pharmaceutically acceptable carrier. A composition is said to be a "pharmaceutically acceptable carrier" if its administration can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable carriers are well-known to those in the art. (See, e.g., Gennaro (ed.), Remington's

Pharmaceutical Sciences (Mack Publishing Company, 19th ed. 1995)) Formulations may further include one or more excipients, preservatives, solubilizers, buffering agents, albumin to prevent protein loss on vial surfaces, etc.

5 The form of the pharmaceutical compositions, the route of administration, the dosage and the regimen naturally depend upon the condition to be treated, the severity of the illness, the age, weight, and sex of the patient, etc.

10 The pharmaceutical compositions of the present invention can be formulated for a topical, oral, parenteral, intranasal, intravenous, intramuscular, subcutaneous or intraocular administration and the like.

15 Typically, 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 water or physiological saline, permit the constitution of injectable solutions.

20 The doses used for the administration can be adapted as a function of various parameters, and in particular as a function of the mode of administration used, of the relevant pathology, or alternatively of the desired duration of treatment.

25 To prepare pharmaceutical compositions, an effective amount of the antibody of the present invention may be dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium.

30 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 of the active compounds 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.

An antibody of the present 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 vegetable 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 compounds in the required amount in the appropriate solvent with various 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.

5 The preparation of more, or highly concentrated solutions for direct injection is also contemplated, where the use of DMSO as solvent is envisioned to result in extremely rapid penetration, delivering high concentrations of the active agents to a small tumor area.

10 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.

15 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, subcutaneous 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. For example, one dosage could be dissolved in 1 ml of isotonic NaCl
20 solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). 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.

25

 The antibodies of the present invention may be formulated within a therapeutic mixture to comprise about 0.0001 to 1.0 milligrams, or about 0.001 to 0.1 milligrams, or about 0.1 to 1.0 or even about 10 milligrams per dose or so. Multiple doses can also be administered.

30

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

In some embodiments, the use of liposomes and/or nanoparticles is contemplated for the introduction of antibodies into host cells. The formation and use of liposomes and/or nanoparticles are known to those of skill in the art.

5

Nanocapsules can generally entrap compounds in a stable and reproducible way. To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μm) are generally designed using polymers able to be degraded in vivo. Biodegradable polyalkyl-cyanoacrylate nanoparticles that meet these requirements are contemplated for use in the present invention, and such particles may be easily made.

10

Liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs)). MLVs generally have diameters of from 25 nm to 4 μm . Sonication of MLVs results in the formation of small unilamellar vesicles (SUVs) with diameters in the range of 200 to 500 $\text{\AA}$, containing an aqueous solution in the core. The physical characteristics of liposomes depend on pH, ionic strength and the presence of divalent cations.

15

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.

20

FIGURES:

25

Figure 1: Effect of OxA, Cetuximab (Cetux), Cetox1 and Cetox2 on the cell growth of pancreas adenocarcinoma cells, AsPC-1 cells. Cells were treated for 48h with 1 μM of each compound and then cells were counted. Results were expressed as the percentage of untreated cell number (Control).

30

Figure 2: Effect of OxA, Cetuximab (Cetux), Cetox1 and Cetox2 on the cell growth of colon adenocarcinoma cells, LoVo cells. Cells were treated for 48h with 1 μM of each compound and then cells were counted. Results were expressed as the percentage of untreated cell number.

Figure 3: Effect of OxB, Cetox1 and Cetox2 on the cell growth of CHO-OX1R/H344A cells expressing H344R OX1R mutant. Substitution of His344 by alanine residue induced the inability of OX1R to recognize orexins. Cells were treated for 48h with 0.1μM of each compound and then cells were counted. Results were expressed as the percentage of untreated cell number (Control).

Figure 4: Effect of OxB, Cetox1 and Cetox2 on the cell growth of stably CHO-OX2R cells expressing recombinant OX2R. Cells were treated for 48h with 0.1μM of each compound and then cells were counted. Results were expressed as the percentage of untreated cell number (Control).

Figure 5: Effect of OxB, Cetox1 and Cetox2 on apoptosis in HEK-OX1R cells expressing recombinant OX1R. SHP-2 protein tyrosine phosphatase inhibitor, NSC-87877, blocks orexin-induced apoptosis. HEK-OX1R cells were challenged with 0.1 μM OxB, Cetox1 or Cetox2 for 48 hr in the absence (white bars) or the presence (black bars) of NSC-87877 (50 μM). Apoptosis was measured by determination of annexin V-PE binding, and results are expressed as the percentage of apoptotic cells

Figure 6: Competitive inhibition of specific ¹²⁵I-OxA binding to HEK-OX1R cells by increasing concentrations of unlabeled OxB, Cetuximab (Cetux), Cetox1 and Cetox2. Cells were incubated with the indicated concentration of OxB (●), Cetux (■), Cetox1 (□) and Cetox2 (▲). Results were expressed as the percentage of radioactivity specifically bound in the absence of added unlabeled compound. Each point is the mean of three separate experiments. ND, not determined

Figure 7: Effect of daily inoculation of OxB (▲), Cetox1 (●) and Cetox2 (△) on the growth of tumors developed by xenografting human PDCA cells in nude mice. AsPC-1 cells were inoculated in the flank of nude mice at day 0. Mice were injected daily intraperitoneally with 100 μl of OxB (20μg) or Cetox1 (200μg) or Cetox2 (200μg) solutions or with 100 μl of PBS (○) for controls. The daily treatment corresponded to 0,275 μmoles of OxB/Kg and 0,051 μmoles of Cetox1 or Cetox2/Kg. After 45 days of treatment, mice were sacrificed and tumor volume and weight were then recorded. The development of tumors was followed by caliper measurement. Data are the means ± SE of 6 tumors in each group. *** p < 0.01 versus control.

Figure 8: Effect of daily inoculation of Cetuximab (cetux), Cetox1 and Cetox2 on the growth of tumors developed by xenografting human colorectal cells in nude mice.

LS-174-T cells were inoculated in the flank of nude mice at day 0. Mice were injected 2 times per week intraperitoneally with 200 μ l of Cetuximab (1000 μ g) or Cetox1 (100 μ g) or Cetox2 (100 μ g) solutions or not injected for controls. The development of tumors was followed by caliper measurement. Data are the means $\pm$ SE of 6 tumors in each group.

EXAMPLES:

EXAMPLE 1:

We produced two types of structures based on the frame of the recombinant anti-EGFR antibody Cetuximab, both on the C-terminus of the light chain of the antibody: the nucleic acid molecule encoding for the first recombinant antibody comprises the sequence SEQ ID NO:2 (named "Cetox 1"), the second building contains the same sequence with C-terminal 3 additional amino acids "GRR" (named "Cetox 2). In our system, the armed antibody obtained by genetic engineering is produced in eukaryotic mammalian cell (CHO) as is its counterpart antibody Cetuximab used clinically, and thus amidation occurs in the host cell during phase of production catalyzed by the enzyme PAM (peptidyl-glycine alpha-amidating mono-oxygenase) actually present in the CHO line (N. Hayashi et al Production of bioactive progastrin from the non-neuroendocrine cell lines CHO and COS-7, FEBS 1994) but is also influenced by carboxypeptidase E-like 2 which cleaves C-terminal arginine (Wulff BS et al. Efficient amidation of C-peptide NPY Precursors deleted by non-endocrine cells is affected by the presence of Lys-Arg at the C -terminus. Mol. Cell Endocrinol. 1993). Adding the sequence GRR C-terminal is intended to permit the maturation of the peptide in the amide form, identical to that produced by chemical synthesis.

In vitro results:

The first results obtained in vitro with these two constructions show that i) Cetox 2 is functional and induces apoptosis in various colon lines expressing the receptor OX1R. The antibody construction Cetox 1 is not functional. The activity of cetuximab is not reduced by adding Orexin B sequence, and even is significantly improved on wild Kras lines (SW48) responsive to cetuximab and on cell lines which are Braf or Kras mutated (#; HT29, SW620, LS174T, LoVo and AsPC1) described as to be resistant to cetuximab.

In vivo results:

We also performed in vivo experiments with the armed antibodies (Cetox 1 and Cetox 2) against 3 different types of xenografts, 2 colon and pancreas 1 that express OX1R and that are mutated for Kras. Our results showed, as observed in the in vitro results, increased efficiency of Cetox 2 while Cetox 1 showed similar efficacy to the unmodified Cetuximab (Cetux). Xenograft established with the colon line LS174T for which the antibody Cetuximab has little activity, when armed with amidated orexin polypeptide shows a gain of efficiency in the inhibition of tumor growth in comparison with Cetuximab used at the same dose. Said results show that the armed antibody renders tumor cells sensitive to apoptosis, regardless of their Kras status. Our technology is thus particularly suitable for the treatment of tumors which are Kras mutated.

EXAMPLE 2:

The inventors produced different constructions of the fusion proteins of the invention. Orexin A and Orexin B are fused to an immunoglobulin chain (heavy chain or light chain) of an antibody (e.g. Cetuximab (Cetux), Rituximab (Ritux) or Trastuzumab (Trast)). The different constructions are depicted in Table 1. The inventors demonstrated that said constructions induce inhibition of HEK-OX1R cell growth (Table 1).

Table 1: Inhibition of cell growth by the fusion protein of the invention.

Results are expressed as the percentage of inhibition of HEK-OX1R cell growth, assuming that untreated cells displays no inhibition (0%). HC, heavy chain; LC, light chain; Ritux, Rituximab; Cetux, Cetuximab ; Trast, Trastuzumab.

Name	Construction	Inhibition of cell growth, %
OxB	RSGPPGLQGRLQRLQASGNHAAGILTM (SEQ ID NO:2)	40
Cetox 1 or C8	Cetux-RSGPPGLQGRLQRLQASGNHAAGILTM (Cetux-SEQ ID NO:2)	30
Cetox 2 or C9	Cetux-RSGPPGLQGRLQRLQASGNHAAGILTMGRR	51

	(Cetux-SEQ ID NO :8)	
C1	Cetux (HC)-RSGPPGLQGRLQRLQASGNHAAGILTMGRR (Cetux (HC)- SEQ ID NO :8)	30
C2	Cetux (LC+HC)-RSGPPGLQGRLQRLQASGNHAAGILTMGRR (Cetux (LC+HC)- SEQ ID NO :8)	37
C3	Cetux (F(ab') ₂)-RSGPPGLQGRLQRLQASGNHAAGILTMGRR (Cetux (F(ab') ₂)- SEQ ID NO :8)	35
C6	Cetux (F(ab))-RSGPPGLQGRLQRLQASGNHAAGILTMGRR (Cetux (F(ab))- SEQ ID NO :8)	26
C10	Cetux-RSGPPGLQGRLQRLQASGNHAAGILTMGR (Cetux-SEQ ID NO :9)	50
C11	Cetux-RSGPPGLQGRLQRLQASGNHAAGILTMG (Cetux-SEQ ID NO :10)	65
C12	Cetux-GLQGRLQRLQASGNHAAGILTMGRR (Cetux-SEQ ID NO :11)	65
C12'	Cetux-GLQGRLQRLQASGNHAAGILTMG (Cetux-SEQ ID NO :12)	68
C14	RSGPPGLQGRLQRLQASGNHAAGILTMGRR-Cetux (SEQ ID NO :8-Cetux)	65
C14'	RSGPPGLQGRLQRLQASGNHAAGILTM-Cetux (SEQ ID NO:2-Cetux)	14
C15	Trast-RSGPPGLQGRLQRLQASGNHAAGILTM (Trast-SEQ ID NO:2)	40
C18	Ritux-RSGPPGLQGRLQRLQASGNHAAGILTM (Ritux-SEQ ID NO:2)	30
C21	Cetux-QPLPDCCRQKTCSCRLYELLHGAGNHAAGILTL (OxA) (Cetux-SEQ ID NO:13)	38

REFERENCES:

5 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 fusion protein which comprises an immunoglobulin chain or a fragment thereof fused to an orexin polypeptide.
2. The fusion protein of claim 1 which consists of an immunoglobulin light chain fused
5 to an orexin polypeptide.
3. The fusion protein of claim 1 which consists of an immunoglobulin heavy chain fused to an orexin polypeptide.
4. The fusion protein of claim 1 wherein the orexin polypeptide is SEQ ID NO:1 or SEQ ID NO:13.
- 10 5. The fusion protein of claim 1 wherein the orexin polypeptide 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:2 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
15 substituted.
6. The fusion protein of claim 1 wherein the orexin polypeptide comprises the amino acid sequence ranging from the amino acid residue at position 10 to the amino acid residue at position 28 in SEQ ID NO:2 wherein at least one amino acid residue position 10, 12, 13, 14, 19, 21 or 23 is substituted and the amino acid residues at
20 position 11; 15; 16; 17; 18; 20; 22; 24; 25; 26; 27; and 28 are not deleted or substituted.
7. The fusion protein of claim 1 wherein the orexin polypeptide is SEQ ID NO:2 or SEQ ID NO:14.
8. The fusion protein of claim 7 wherein the methionine residue at position 28 is
25 amidated.
9. The fusion protein of claim 1 wherein the orexin polypeptide is extended at its c-terminal end by at least one amino acid such as a glycine (G).

10. The fusion protein of claim 1 wherein the orexin polypeptide is extended at its c-terminal end by at least 2 amino acids such as GR or GK.
11. The fusion protein of claim 1 wherein the orexin polypeptide is extended at its c-terminal end by at least 3 amino acids such as GRR, GRK, GKR, or GKK.
- 5 12. The fusion protein of claim 1 wherein the orexin polypeptide comprises or consists of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12.
13. The fusion protein of claim 1 wherein the immunoglobulin chain is fused at its C-terminal end to the N-terminal end of the orexin polypeptide.
- 10 14. The fusion protein of claim 1 wherein the immunoglobulin chain is fused at its N-terminal end to the C-terminal end of the orexin polypeptide extended by the amino acid sequence GRR, GRK, GKR, or GKK.
15. The fusion protein of claim 1 which consists of the amino acid sequence as set forth in SEQ ID NO:3:
- 15 16. The fusion protein of claim 15 wherein the last methionine residue of SEQ ID NO: 3 is amidated.
17. A nucleic acid molecule encoding the fusion protein claim 1 which comprises a first nucleic acid sequence encoding for the immunoglobulin chain or the fragment thereof operably linked to a second nucleic acid sequence encoding for the orexin polypeptide.
- 20 18. A vector comprising the nucleic acid of claim 17.
19. A host cell which has been transfected, infected or transformed by the nucleic acid molecule of claim 17 and/or the vector of claim 18.
20. An antibody which comprises at least one fusion protein of claim 1.
21. The antibody of claim 20 which specific for EGFR.
- 25 22. The antibody of claim 20 which is cetuximab.
23. The antibody of claim 20 which comprises the fusion protein of claim 15.

24. The antibody of claim 20 for use as a medicament.
25. A method of treating cancer in a subject in need thereof comprising administering the subject with a therapeutically effective amount of the antibody of claim 20.
26. A method of treating a metastatic colorectal cancer associated with a RAS or BRAF mutation in a subject in need thereof comprising administering a therapeutically effective amount of the antibody of claim 22.
27. A pharmaceutical composition which comprises the antibody of claim 20.

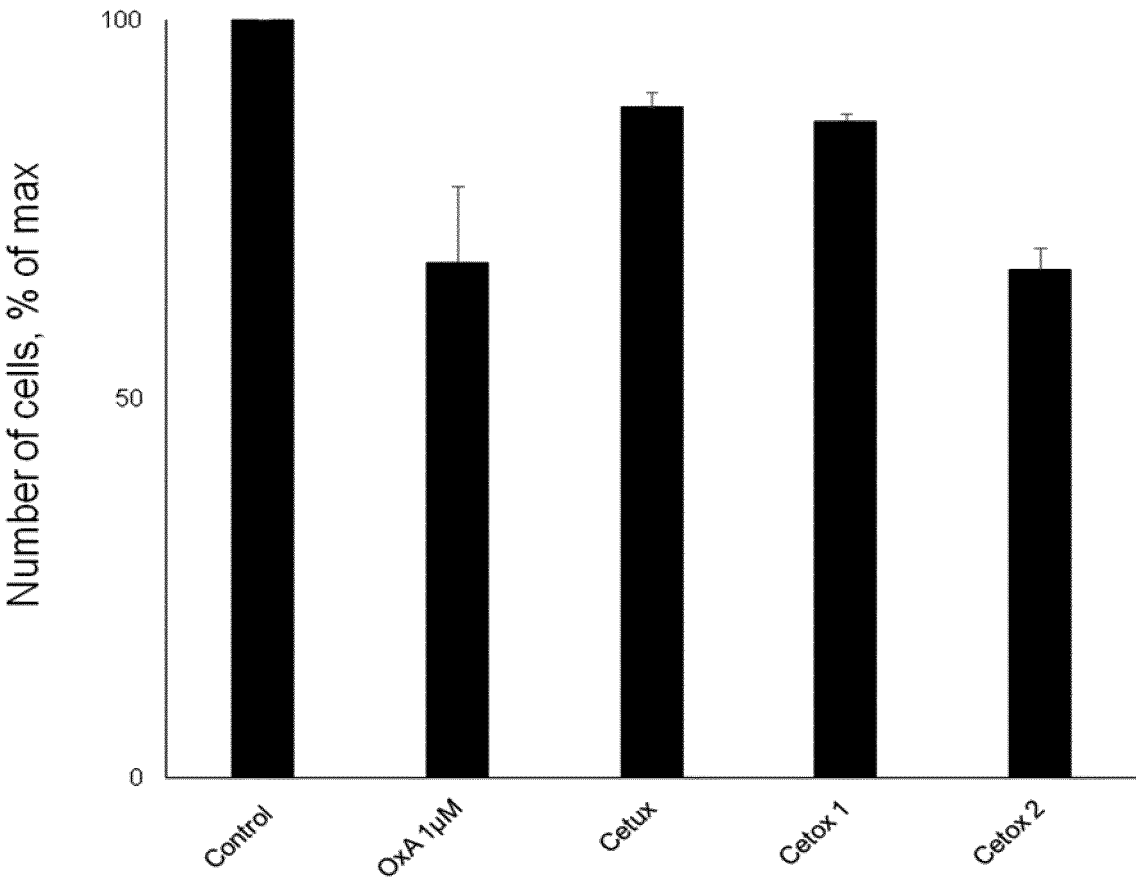


Figure 1

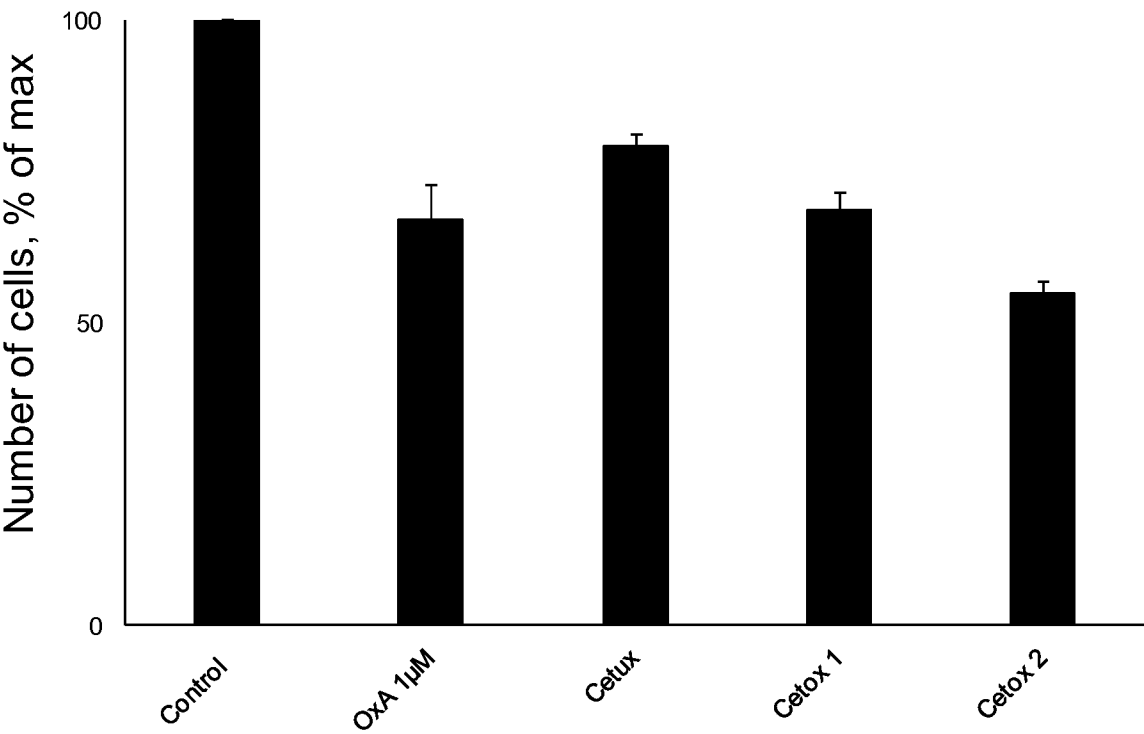


Figure 2

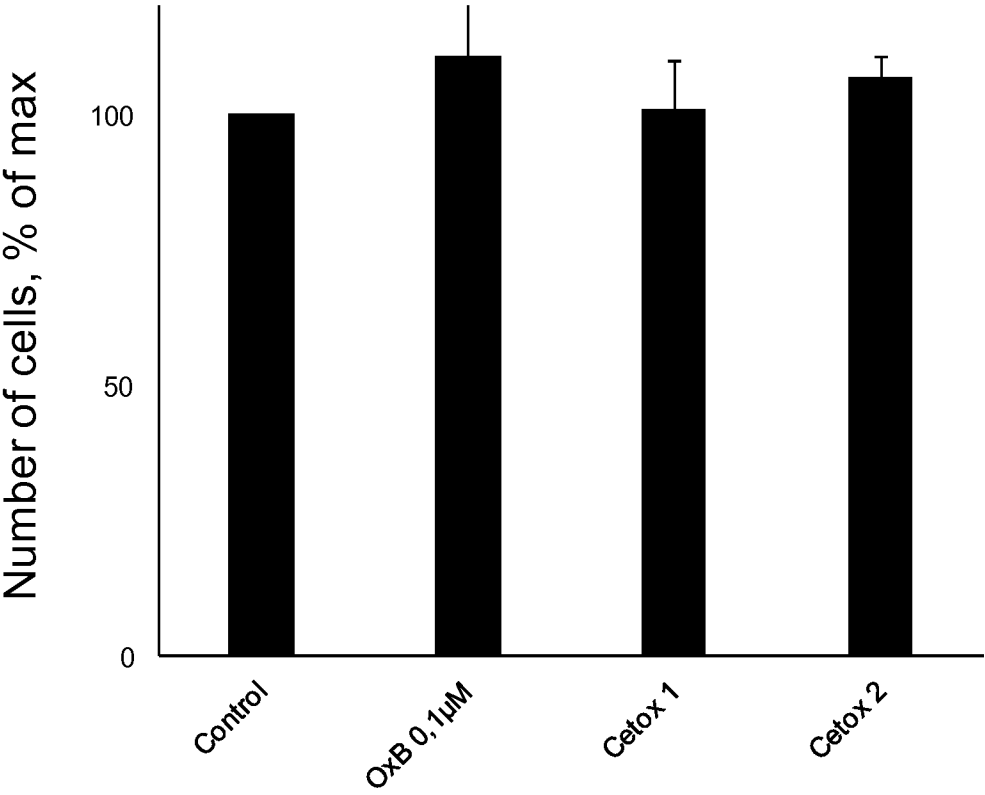


Figure 3

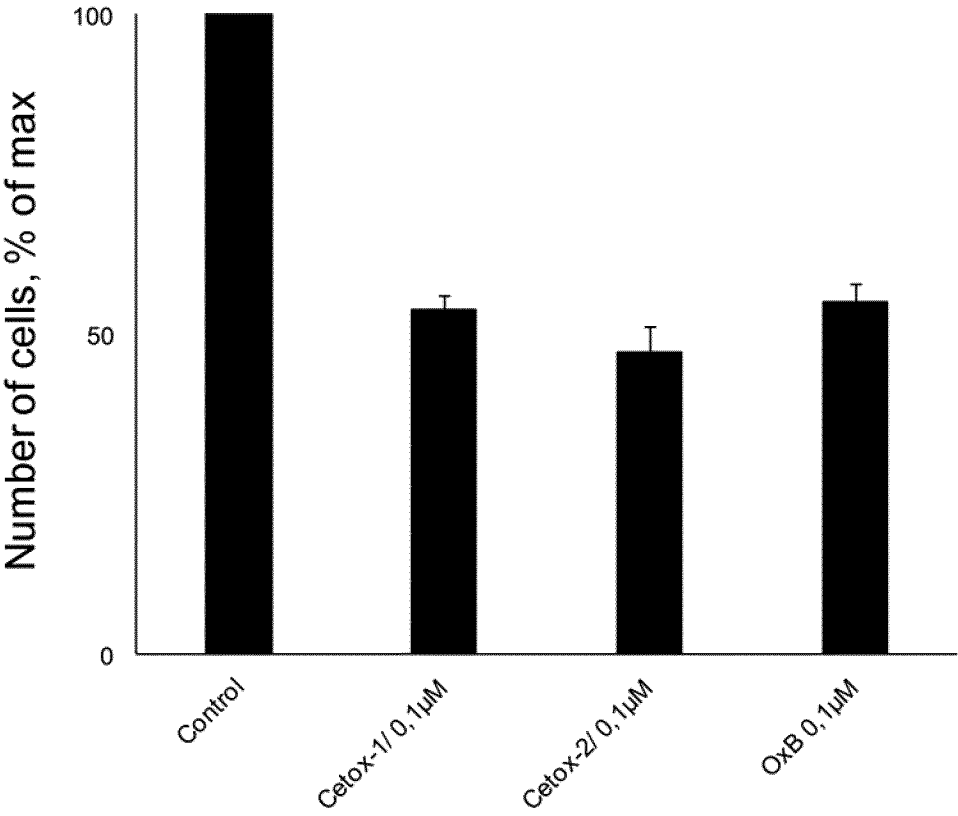


Figure 4

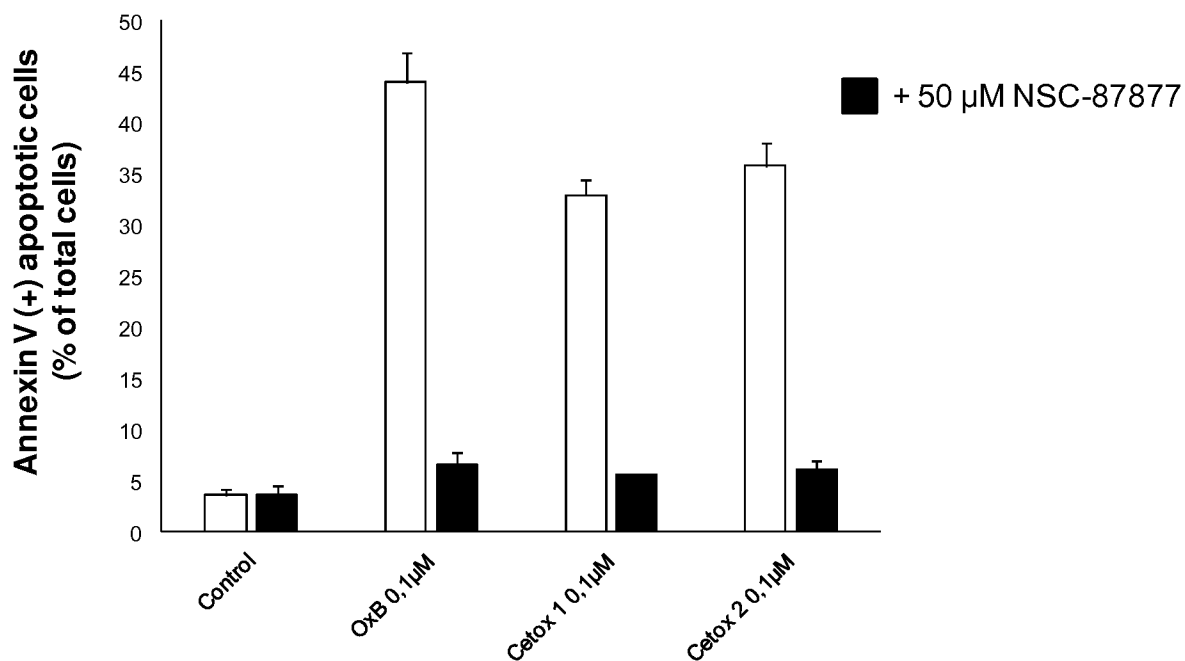


Figure 5

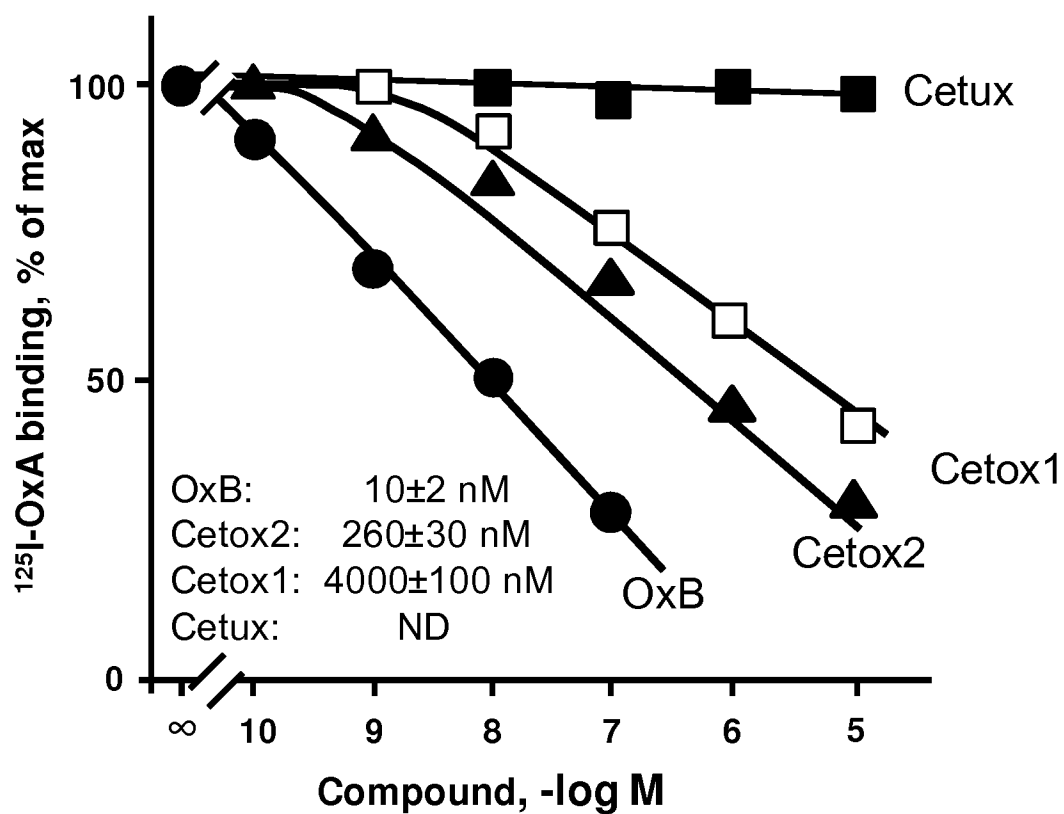


Figure 6

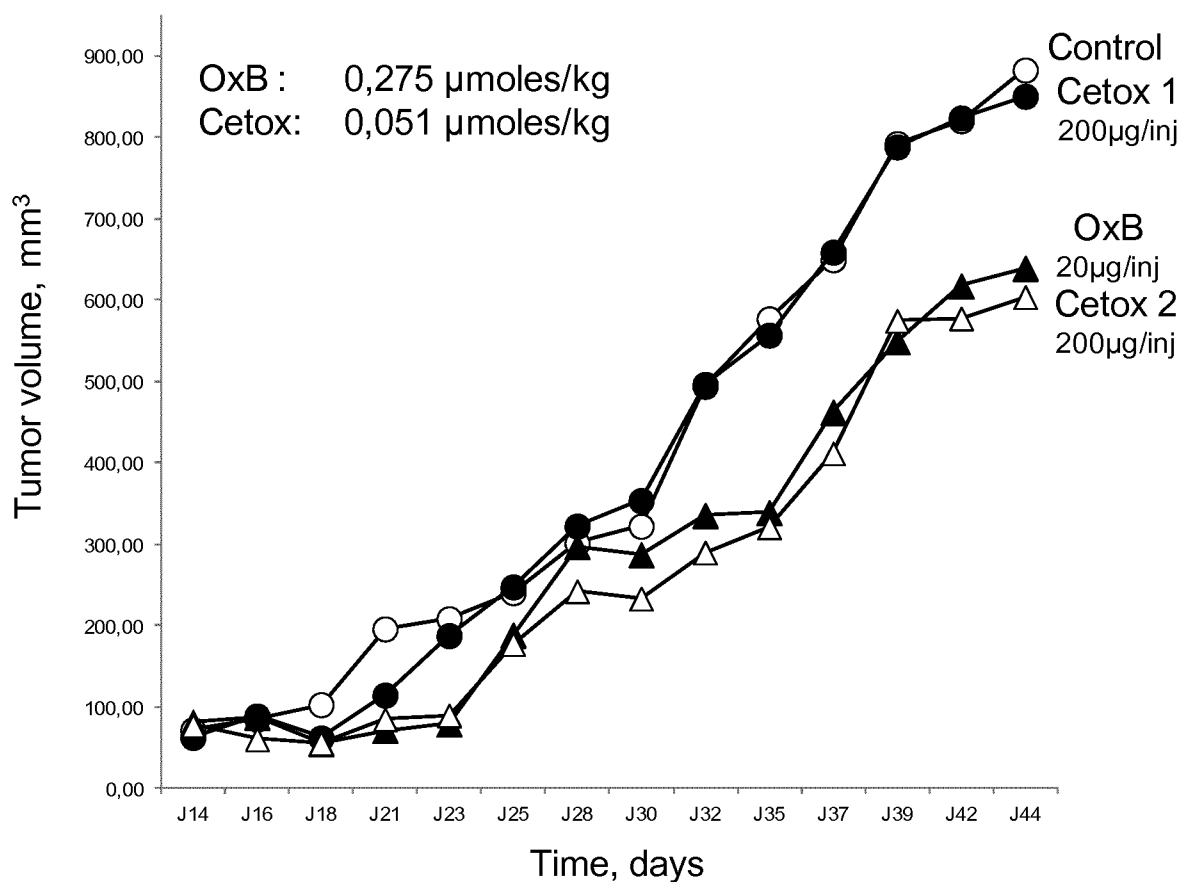


Figure 7

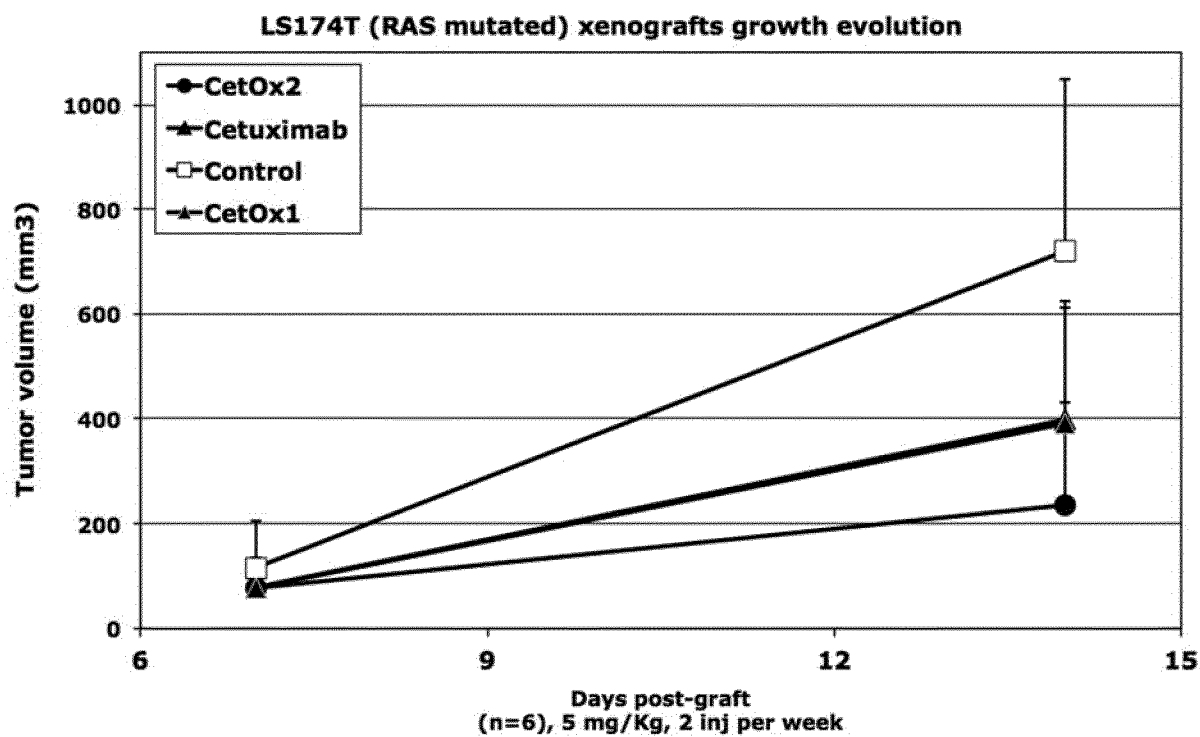


Figure 8

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2016/053475

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. ☒ forming part of the international application as filed:
- ☒ in the form of an Annex C/ST.25 text file.
- ☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
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2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/053475

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/575 C07K16/28
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

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, 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.
X	YANG XUANMING ET AL: "Targeting the Tumor Microenvironment with Interferon-[beta] Bridges Innate and Adaptive Immune Respo", CANCER CELL, CELL PRESS, US, vol. 25, no. 1, 13 January 2014 (2014-01-13), pages 37-48, XP028808992, ISSN: 1535-6108, DOI: 10.1016/J.CCR.2013.12.004 page 38, right-hand column, paragraph 2 - page 4, left-hand column, line 2 page 46 left-hand column "Production of Ab-INFbeta Fusion Protein" -----	1-27
X	WO 2009/068649 A2 (GLAXO GROUP LTD [GB]; ASHMAN CLAIRE [GB]; BATUWANGALA THIL [GB]; BURDE) 4 June 2009 (2009-06-04) example 17; sequence 138 ----- -/-	1-27

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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Date of the actual completion of the international search

2 June 2016

Date of mailing of the international search report

13/06/2016

Name and mailing address of the ISA/

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Authorized officer

Bladier, Cecile

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2016/053475

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/164427 A1 (BIOCON LTD [IN]; GOVINDAPPA NAGARAJ [IN]; SOARES MARIA MELINA [IN]; SA) 9 October 2014 (2014-10-09) claim 29; sequence 28 -----	1-22
X	T.-H. SHIN ET AL: "Enhancement of the Tumor Penetration of Monoclonal Antibody by Fusion of a Neuropilin-Targeting Peptide Improves the Antitumor Efficacy", MOLECULAR CANCER THERAPEUTICS, vol. 13, no. 3, 16 January 2014 (2014-01-16), pages 651-661, XP055196026, ISSN: 1535-7163, DOI: 10.1158/1535-7163.MCT-13-0748 the whole document -----	1-27
A	MARC LABURTHE ET AL: "The orexin receptor OX1R in colon cancer: a promising therapeutic target and a new paradigm in G protein-coupled receptor signalling through ITIMs", BRITISH JOURNAL OF PHARMACOLOGY, vol. 165, no. 6, 22 February 2012 (2012-02-22), pages 1678-1687, XP055196628, ISSN: 0007-1188, DOI: 10.1111/j.1476-5381.2011.01510.x page 1679, left-hand column, paragraph 1 -----	1-27
A	ALAIN COUVINEAU ET AL: "Abstract 5299: The C-terminus of orexin plays a crucial role in the cellular apoptosis induction mediated by OX1 receptor", CANCER RESEARCH, vol. 74, no. 19 Supplement, 30 September 2014 (2014-09-30), pages 5299-5299, XP055196665, ISSN: 0008-5472, DOI: 10.1158/1538-7445.AM2014-5299 the whole document -----	1-22

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2016/053475

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