ANTI CXCR4 ANTIBODIES AND THEIR USE FOR THE TREATMENT OF CANCER

The present invention relates to novel antibodies, in particular murine monoclonal antibodies, chimeric and humanized, able to bind specifically to chemokine receptors (CXCR) as well as the amino and nucleic acid sequences coding for such antibodies. From one aspect, the invention relates to novel antibodies, derived compounds or functional fragments, able to bind specifically to the CXCR4 and having strong anti-tumor activities. The invention also comprises the use of such antibodies as a drug for the preventive and/or therapeutic treatment of cancer, as well as in the procedures or kits related to cancer diagnosis. Finally, the invention comprises compositions comprising such antibodies in combination or conjugation with other anti-cancer compounds, such as antibodies, toxins, cytotoxic/cytostatic, and the use of same for the prevention and/or treatment of certain cancers.

Chemokines are small, secreted peptides that control the migration of leukocytes along a chemical gradient of ligand, known as chemokine gradient, especially during immune reactions (Zlotnick A. et al., 2000). They are divided into two major subfamilies, CC and CXC, based on the position of their NH_2-terminal cysteine residues, and bind to G protein coupled receptors, whose two major sub families are designated CCR and CXCR. More than 50 human chemokines and 18 chemokine receptors have been discovered so far.

Many cancers have a complex chemokine network that influences the immune-cell infiltration of tumor, as well as tumor cell growth, survival, migration and angiogenesis. Immune cells, endothelial cells and tumor cells themselves express chemokine receptors and can respond to chemokine gradients. Studies of human cancer biopsy samples and mouse cancer models show that cancer cell chemokine-receptor expression is associated with increase metastatic capacity. Malignant cells from different cancer types have different profiles of chemokine-receptor expression, but Chemokine receptor 4 (CXCR4) is most commonly found. Cells from at least 23 different types of human cancers of epithelial, mesenchymal and haematopoietic origin express CXCR4 receptor (Balkwill F. et al., 2004).

Chemokine receptor 4 (also known as fusin, CD184, LESTR or HUMSTR) exists as two isoforms comprising 352 or 360 amino acids. Residue Asn11 is glycosylated, residue Tyr21 is modified by the addition of a sulfate group and Cys 109 and 186 are bond with a disulfide bridge on the extracellular part of the receptor (Juarez J. et al., 2004).

This receptor is expressed by different kind of normal tissues, naïve, non-memory T-cells, regulatory T cells, B-cells, neutrophils, endothelial cells, primary monocytes, dendritic cells, Natural Killer cells, CD34+ hematopoietic stem cells and at a low level in heart, colon, liver, kidneys and brain. CXCR4 plays a key role in leukocytes trafficking, B cell lymphopoiesis and myelopoiesis.

CXCR4 receptor is over-expressed in a large number of cancers including but not limited to colon (Ottaiano A. et al., 2004), breast (Kato M. et al., 2003), prostate (Sun Y.X. et al., 2003), lung [small-cell- and non-small-cell- carcinoma (Phillips R.J. et al., 2003)], ovary (Scotton C.J. et al., 2002), pancreas (Koshiba T. et al., 2000), kidneys, brain (Barbero S et al., 2002), glioblastoma and lymphomas.

The unique ligand of CXCR4 receptor described so far is the Stromal-cell-Derived Factor-1 (SDF-1) or CXCL12. SDF-1 is secreted in large amount in lymph node, bone marrow, liver, lung and to a less extent by kidneys, brain and skin. CXCR4 is also recognized by an antagonistic chemokine, the viral macrophage inflammatory protein II (vMIP-II) encoded by human herpesvirus type III.

CXCR4/SDF-1 axis plays a key role in cancer and is implicated directly in migration, invasion leading to metastases. Indeed, cancer cells express CXCR4 receptor, they migrate and enter the systemic circulation. Then cancer cells are arrested in vascular beds in organs that produce high levels of SDF-1 where they proliferate, induce angiogenesis and form metastatic tumors (Murphy PM., 2001). This axis is also involved in cell proliferation via activation of Extracellular-signal-Regulated Kinase (ERK) pathway (Barbero S. et al., 2003) and angiogenesis (Romagnani P., 2004). Indeed, CXCR4 receptor and its ligand SDF-1 clearly promote angiogenesis by stimulating VEGF-A expression which in turns increases expression of CXCR4/SDF-1 (Bachelder R.E. et al., 2002). It is also known that tumor associated macrophages (TAM) accumulated in hypoxic areas of tumors and are stimulated to co-operate with tumor cells and promote angiogenesis. It was observed that hypoxia up-regulated

selectively expression of CXCR4 in various cell types including TAM (Mantovani A. et al., 2004). It has been recently demonstrated that CXCR4/SDF-1 axis regulates the trafficking/homing of CXCR4+ hematopoietic stem/progenitor cells (HSC) and could play a role in neovascularization. Evidence indicates that besides HSC, functional CXCR4 is also expressed on stem cells from other tissues (tissue-committed stem cells = TCSCs) so SDF-1 may play a pivotal role in chemottracting CXCR4+ TCSCs necessary for organ/tissue regeneration but these TCSC may also be a cellular origin of cancer development (cancer stem cells theory). A stem cell origin of cancer was demonstrated for human leukemia and recently for several solid tumors such as brain and breast. There are several examples of CXCR4+ tumors that may derive from the normal CXCR4+ tissue/organ-specific stem cells such as leukemias, brain tumors, small cell lung cancer, breast cancer, hepatoblastoma, ovarian and cervical cancers (Kucia M. et al., 2005).

Targeting cancer metastases by interfering with CXCR4 receptor was demonstrated *in vivo* using a monoclonal antibody directed against CXCR4 receptor (Muller A. et al., 2001). Briefly, it was shown that a monoclonal antibody directed against CXCR4 receptor (Mab 173 R&D Systems) decreased significantly the number of lymph node metastases in an orthotopic breast cancer model (MDA-MB231) in SCID mice. Another study (Phillips R.J et al., 2003) also showed the critical role of SDF-1/CXCR4 axis in metastases in an orthotopic lung carcinoma model (A549) using polyclonal antibodies against SDF-1 but in this study there was no effect neither on tumor growth nor on angiogenesis. Several other studies described also the inhibition of either metastasis *in vivo* using siRNAs duplexes of CXCR4 (Liang Z. et al., 2005) biostable CXCR4 peptide antagonists (Tamamura H. et al., 2003) or tumor growth *in vivo* using small molecule antagonist of CXCR4 like AMD 3100 (Rubin J.B. et al., 2003; De Falco V. et al., 2007) or Mab (patent WO2004/059285 A2). Thus, CXCR4 is a validated therapeutic target for cancers.

Chemokine receptor 2 (CXCR2), another chemokine receptor is also described as an interesting target in oncology. Indeed, CXCR2 transmits an autocrine cell growth signal in several tumor cell types and can also affect tumor growth indirectly by promoting angiogenesis (Tanaka T. et al. 2005).

CXCR2 chemokine receptor encompasses 360 amino acids. It is expressed mainly in endothelial cells and specially during neovascularization. Several chemokines bind CXCR2 receptor: CXCL5, -6, -7, IL-8, GRO-α, -β and γ which belong to ERL+ pro-angiogenic chemokines. The CXCR2 receptor share sequence homologies with CXCR4 receptor: 37% sequence identity and 48% sequence homology. The CXCR2/ligands axis is involved in several tumor growth mechanisms such as metastasis (Singh RK. et al., 1994) cell proliferation (Owen J.D. et al., 1997) and in ERL+ chemokines-mediated angiogenesis (Strieter R.M. et al., 2004; Romagnani et al., 2004). Finally, tumor-associated macrophages and neutrophils are key elements of inflammatory-induced tumor growth and chemokines such as CXCL5, IL-8 and GRO-α initiate neutrophils recruitment.

Dimerization has emerged as a common mechanism for regulating the function of G-protein-coupled receptors, among these are chemokine receptors (Wang J. and Norcross M., 2008). Homo- and heterodimerization in response to chemokine binding has been shown to be required for the initiation and the alteration of signaling by a number of chemokine receptors. Growing evidence supports the concept that receptor dimers or oligomers are probably the basic functional unit of chemokine receptors. Chemokine receptor dimers are found in the absence of ligands and chemokines induce conformational changes of receptor dimers. CXCR4 is known to form homodimers but also heterodimers, for examples with the δ-opioid receptor (DOR) (Hereld D., 2008) or CCR2 (Percherancier Y. et al., 2005). In the latter example, peptides derived from the transmembrane domains of CXCR4 inhibited activation by blocking the ligand-induced conformational transitions of the dimer (Percherancier Y. et al., 2005). Another study showed that CXCR4-TM4 peptide, a synthetic peptide of the transmembrane region of CXCR4, decreases energy transfer between protomers of CXCR4 homodimers and inhibits SDF-1-induced migration and actin polymerization in malignant cells (Wang J. et al., 2006). More recently, it was also described that CXCR7 formed functional heterodimers with CXCR4 and enhanced SDF-1-induced signaling (Sierro F. et al., 2007). Other examples of constitutive heterodimers include studies showing CXCR1 and CXCR2 interact as well as forming respective homodimers. No interactions were noted for either of them with another GPCR (alpha(1A)-adrenoreceptor), indicating the specificity of CXCR1 and CXCR2 interaction (Wilson S. et al., 2005).

As previously mentioned, CXCR4 and CXCR2 receptors are interesting tumor targets. Interfering with those receptors should inhibit tumor growth and metastases in a very efficient way, by decreasing tumor cell proliferation, angiogenesis, tumor cell migration and invasion, neutrophils and macrophages recruitment by tumors and by inhibiting CXCR4 cancer stem cells.

One of the inventive aspects of the present invention is to generate a mouse monoclonal antibody inducing CXCR4 dimers conformational changes. The invention encompasses a CXCR4 Mab 414H5 (or fragments thereof) able to bind and to induce conformational changes of both CXCR4 homodimers and CXCR4/CXCR2 heterodimers, and having strong anti-tumor activities both in mice xenograft and survival models. The invention also encompasses a CXCR4 Mab 515H7 (or fragments thereof) able to bind and to induce conformational changes of both CXCR4 homodimers and CXCR4/CXCR2 heterodimers, and having strong anti-tumor activities. Anti-CXCR4 414H5 Mab inhibits tumor growth in MDA-MB-231 xenograft model and increases mice survival in U937 model. They induce conformational changes on CXCR4 homodimers but also on CXCR4/CXCR2 heterodimers. This new property should be of interest for cancer therapy application given the important roles of these two chemokine receptors in cancer.

Targeting both homo- and hetero-dimers of receptors has already been found to increase Mab therapeutic effect. Indeed, it has been demonstrated for example, that a Mab (h7C10) targeting both IGF-1R and insulin/IGF-1 hybrid receptors was more potent to inhibit tumor growth *in vivo* than a Mab targeting solely IGF-1R (Pandini G., 2007).

Moreover the anti-CXCR4 Mabs 414H5 and 515H7 are silent antagonists for CXCR4, they do not change basal signal in *in vitro* assays but inhibit signaling induced by SDF-1 in different assays (GTPγS binding, cAMP release) and are also able to inhibit SDF-1 induced tumor cells proliferation and migration *in vitro*.

Molecules acting as either partial agonists or inverse agonists exhibit intrinsic activity in the absence of ligands. These types of molecules stabilize, respectively a high-affinity or a low-affinity GPCR state, even in the absence of ligand, thereby activating or inhibiting downstream signaling cascades (Galandin et al., 2007; Kenakin, 2004).

In case of 414H5 and 515H7 Mabs, these molecules behaved as silent antagonists, without any intrinsic activity at CXCR4 receptor in the absence of SDF-1. This pharmacological feature is likely to be associated with less adverse side-effects as compared to partial or inverse agonists, as already observed for opioid receptor ligands (Bosier and Hermans, 2007). Indeed, the functional activity of both 414H5 and 515H7 Mabs is totally dependent on the presence of SDF-1 and no modulation of CXCR4 receptor activity will be observed in tissues and organs where SDF-1 ligand is not expressed, secreted or provided by the blood flow. Thus, 414H5 and 515H7 Mabs are likely to be less toxic as compared to other CXCR4 receptor ligands with positive or negative efficacy. In addition, silent antagonists are the minority species in the pharmacological space (Wurch et al., 1999, Kenakin, 2004).

Surprisingly, for the first time, inventors have managed to generate antibodies capable of binding to CXCR4 but also capable of inducing conformational changes of the CXCR4 homodimers and/or heterodimers. More particularly, the antibodies of the invention are capable of inducing conformational changes of the CXCR4 homodimers but also of the CXCR4/CXCR2 heterodimers.

In the following specification, the plural expression "CXCR4 dimers" must be understood as encompassing the CXCR4 homodimers and also the CXCR4/CXCR2 heterodimers.

It must be mentioned at this stage that such antibodies have never been described in the prior art. Moreover, it must be mentioned that the existence of CXCR4/CXCR2 heterodimers was never described.

A part of the invention is the discovery of the existence of a heterodimer formed by CXCR4 and CXCR2.

So, in a particular embodiment, the present disclosure is directed to an isolated complex comprising or consisting of the CXCR4/CXCR2 heterodimer.

Preferably, CXCR4 compound part of said CXCR4/CXCR2 heterodimer complex is one of the two human CXCR4 isoforms selected from the group consisting of:

- the chemokine (C-X-C motif) receptor 4 isoform b [Homo sapiens] having the sequence as depicted under the Genbank accession number NP_003458 SEQ ID No. 29:

MEGISIYTSDNYTEEMGSGDYDSMKEPCFREENANFNKIFLPTIYSIIFLTGIVGN

GLVILVMGYQKKLRSMTDKYRLHLSVADLLFVITLPFWAVDAVANWYFGNFL
CKAVHVIYTVNLYSSVLILAFISLDRYLAIVHATNSQRPRKLLAEKVVYVGVWI
PALLLTIPDFIFANVSEADDRYICDRFYPNDLWVVVFQFQHIMVGLILPGIVILSC
YCIIISKLSHSKGHQKRKALKTTVILILAFFACWLPYYIGISIDSFILLEIIKQGCEFE
NTVHKWISITEALAFFHCCLNPILYAFLGAKFKTSAQHALTSVSRGSSLKILSKG
KRGGHSSVSTESESSSFHSS;

- the chemokine (C-X-C motif) receptor 4 isoform a [Homo sapiens] having the sequence as depicted under the Genbank accession number NP_001008540 SEQ ID No. 30:

MSIPLPLLQIYTSDNYTEEMGSGDYDSMKEPCFREENANFNKIFLPTIYSIIFLTGI
VGNGLVILVMGYQKKLRSMTDKYRLHLSVADLLFVITLPFWAVDAVANWYFG
NFLCKAVHVIYTVNLYSSVLILAFISLDRYLAIVHATNSQRPRKLLAEKVVYVG
VWIPALLLTIPDFIFANVSEADDRYICDRFYPNDLWVVVFQFQHIMVGLILPGIVI
LSCYCIIISKLSHSKGHQKRKALKTTVILILAFFACWLPYYIGISIDSFILLEIIKQGC
EFENTVHKWISITEALAFFHCCLNPILYAFLGAKFKTSAQHALTSVSRGSSLKILS
KGKRGGHSSVSTESESSSFHSS;

- an alternate transcriptional splice variant or a natural variant thereof having at least 95 % identity with one of these b or a isoforms having the SEQ ID No. 29 or 30; and
- a fragment thereof capable of being specifically recognizing by its natural ligand stromal cell-derived factor-1 (SDF-1) and having preferably at least 100, 150 and 200 amino acid length.

Preferably, CXCR2 compound part of said CXCR4/CXCR2 heterodimer complex is selected from the group consisting of:

- the interleukin 8 receptor beta [Homo sapiens] having the sequence as depicted under the Genbank accession number NP_001548 SEQ ID No. 31:

MEDFNMESDSFEDFWKGEDLSNYSYSSTLPPFLLDAAPCEPESLEINKYFVVIIY
ALVFLLSLLGNSLVMLVILYSRVGRSVTDVYLLNLALADLLFALTLPIWAASKV
NGWIFGTFLCKVVSLLKEVNFYSGILLLACISVDRYLAIVHATRTLTQKRYLVKF
ICLSIWGLSLLLALPVLLFRRTVYSSNVSPACYEDMGNNTANWRMLLRILPQSF
GFIVPLLIMLFCYGFTLRTLFKAHMGQKHRAMRVIFAVVLIFLLCWLPYNLVLL
ADTLMRTQVIQETCERRNHIDRALDATEILGILHSCLNPLIYAFIGQKFRHGLLKI
LAIHGLISKDSLPKDSRPSFVGSSSGHTSTTL;

- an alternate transcriptional splice variant or a natural variant thereof having at least 95 % identity with this interleukin 8 receptor beta having the SEQ ID No. 31; and
- a fragment thereof capable of being specifically recognizing by IL-8 and having preferably at least 100, 150 and 200 amino acid length.

In this particular embodiment, the present disclosure also comprises an isolated RNA or DNA encoding for a polypeptide comprising said CXCR4/CXCR2 heterodimer complex.

This disclosure further comprises a nucleic construct, preferably an expression vector, such as a plasmid, encoding said CXCR4/CXCR2 heterodimer complex.

The disclosure further comprises a composition comprising at least one nucleic construct, preferably an expression vector, such as a plasmid, encoding the part CXCR4 of said CXCR4/CXCR2 heterodimer complex, and a second construct, preferably an expression vector, such as a plasmid, encoding the part CXCR2 of said CXCR4/CXCR2 heterodimer complex.

In this embodiment, the disclosure further comprises a method for the preparation of a recombinant host cell which expresses said CXCR4/CXCR2 heterodimer complex, wherein this method comprises a step of transforming said host cell:

a) with a nucleic construct, preferably an expression vector, such as a plasmid, encoding said CXCR4/CXCR2 heterodimer complex; or

b) with at least one nucleic construct, preferably an expression vector, such as a plasmid, encoding the part CXCR4 of said CXCR4/CXCR2 heterodimer complex, and a second construct, preferably an expression vector, such as a plasmid, encoding the part CXCR2 of said CXCR4/CXCR2 heterodimer complex.

In a preferred embodiment, said host cell is an eukaryotic cell, such as a mammalian cell.

In a preferred embodiment, the nucleic construct(s) encoding said CXCR4/CXCR2 heterodimer complex encode also for a first marker which is associated (particularly by covalent coupling) with the CXCR4 sequence, such as the luc marker, and for a second marker which is associated (particularly by covalent coupling) with the CXCR2 sequence, such as the GFP marker (i.e.for BRET analysis).

The disclosure also comprises a method for selecting a compound having an anti-cancer activity or which can be used for the preparation of a composition for the treatment of cancer, characterized in that said method comprises the step of:

a) contacting a recombinant host cell disclosed herein which expresses said CXCR4/CXCR2 heterodimer complex, with the compound to be tested; and

b) determining whether this compound is capable of modulating, preferably inhibiting, the activity of this CXCR4/CXCR2 heterodimer complex in the recombinant host cell.

In an embodiment, a subject of the present disclosure is a process for the generation and the selection of antibodies disclosed herein.

More particularly, the disclosure concerns a process for the selection of an anti CXCR4 antibody, or one of its functional fragments or derivatives, capable to inhibit both ligand-dependent and ligand-independent activation of CXCR4, said process comprising the following steps:

i) screening the generated antibodies and selecting antibodies capable to bind specifically to CXCR4 and also to modulate CXCR4 activation;

ii) testing the selected antibodies of step i) and selecting antibodies capable to induce CXCR4 homodimers conformational change, and then

iii) testing the selected antibodies of step ii) and selecting antibodies capable to induce CXCR4/CXCR2 heterodimers conformational change.

By the expression "to modulate", it must be understood an increase or an inhibition. Preferably, the selected antibodies of the disclosure must inhibit the CXCR4 activation.

As it was explained before, the induction of CXCR4 dimers conformational changes is a capital aspect of the invention as such antibodies will present a real interest for a larger population of patients.

The generation of the antibody can be realized by any method known by the man skilled in the art, such as for example, fusion of a myeloma cell with spleen cells from immunized mice or other species compatible with the selected myeloma cells [Kohler & Milstein, 1975, Nature, 256:495-497]. The immunized animals could include transgenic mice with human immunoglobulin loci which then directly produce human

antibodies. Another possible embodiment could consist in using phage display technologies to screen libraries.

The screening step i) can be realized by any method or process known by the man skilled in the art. As non limitative examples, can be mentioned ELISA, BIAcore, immunohistochemistry, FACS analysis and functional screens. A preferred process consists in a screen by FACS analysis on CXCR4 transfectant and on at least a tumoral cell line to be sure that the produced antibodies will be able to also recognize the native receptor on tumor cells. This process will be described more precisely in the following examples.

By the expression "to modulate CXCR4 activation" it is intended to modulate at leas one of the activity depicted in the examples 4, 5, 7 and 13 below:

Preferably to modulate:

- The specific binding at cellular membranes of the ligand SDF-1 on the receptor CXCR4 (see example 4), particularly by competition on eukaryotic transformed cell membrane, such as CHO-K1 membranes, stably expressing human wild type CXCR4 receptor;
- The specific binding at cellular membranes of the GTPγS on the receptor CXCR4 (see example 5), particularly on eukaryotic transformed cells membrane, such as NIH-3T3 cells, stably and constitutively expressing wild-type CXCR4 receptor membranes;
- The CXCR4-mediated inhibition of cAMP production (see Example 7); and
- The CXCR4 receptor-mediated mobilization of intracellular calcium stores (see Example 13).

More preferably, this modulation of at least one of these activities is an inhibition of the activity.

In a preferred embodiment of the steps ii) and iii) of selection of the process of the invention, said steps ii) and iii) consist in evaluating antibodies by BRET analysis on cells expressing CXCR4-RLuc/CXCR4-YFP and CXCR4-Rluc/CXCR2-YFP, respectively, and selecting antibodies capable to inhibit at least 40 %, preferably 45 %, 50 %, 55 % and most preferably 60 % of the BRET signal.

The technology BRET is a technology known as being representative of the protein dimerization [Angers et al., PNAS, 2000, 97:3684-89].

The technology BRET, used in the steps ii) and iii) of the process, is well known by the man skilled in the art and will be detailed in the following examples. More particularly, BRET (Bioluminescence Resonance Energy Transfer) is a non-radiative energy transfer occurring between a bioluminescent donor (Renilla Luciferase (Rluc)) and a fluorescent acceptor, a mutant of GFP (Green Fluorescent Protein) or YFP (Yellow fluorescent protein). In the present case EYFP (Enhanced Yellow Fluorescent Protein) was used. The efficacy of transfer depends on the orientation and the distance between the donor and the acceptor. Then, the energy transfer can occur only if the two molecules are in close proximity (1-10 nm). This property is used to generate protein-protein interaction assays. Indeed, in order to study the interaction between two partners, the first one is genetically fused to the Renilla Luciferase and the second one to the yellow mutant of the GFP. Fusion proteins are generally, but not obligatory, expressed in mammalian cells. In presence of its membrane permeable substrate (coelenterazine), Rluc emits blue light. If the GFP mutant is closer than 10 nm from the Rluc, an energy transfer can occur and an additional yellow signal can be detected. The BRET signal is measured as the ratio between the light emitted by the acceptor and the light emitted by the donor. So the BRET signal will increase as the two fusion proteins are brought into proximity or if a conformational change brings Rluc and GFP mutant closer.

If the BRET analysis consists in a preferred embodiment, any method known by the man skilled in the art can be used to measure CXCR4 dimers conformational changes. Without limitation, the following technologies can be mentioned: FRET (Fluorescence Resonance Energy Transfer), HTRF (Homogenous Time resolved Fluorescence), FLIM (Fluorescence Lifetime Imaging Microscopy) or SW-FCCS single wavelength fluorescence cross-correlation spectroscopy).

Other classical technologies could also be used, such as Co-immunoprecipitation, Alpha screen, Chemical cross-linking, Double-Hybrid, Affinity Chromatography, ELISA or Far western blot.

In a particular embodiment of the process according to the disclosure, step ii) consists in evaluating antibodies by BRET analysis on cells expressing both CXCR4-RLuc/CXCR4-YFP and selecting antibodies capable to inhibit at least 40 %, of the BRET signal.

In another particular embodiment of the disclosed process, step iii) consists in evaluating antibodies by BRET analysis on cells expressing both CXCR4-RLuc/CXCR2-YFP and selecting antibodies capable to inhibit at least 40 %, of the BRET signal.

In an embodiment, a subject of the disclosure is an isolated antibody, or one of its functional fragments or derivatives, being obtained by said process. Said antibody or one of its said fragments or derivatives, is capable of binding specifically to the human CXCR4 and, if necessary, preferably moreover capable of inhibiting the natural attachment of its ligand, said antibody being also capable to induce CXCR4 dimers conformational changes.

The expressions "functional fragments and derivatives" will be defined in details later in the present specification.

It must be understood here that the disclosure does not relate to the antibodies in natural form, that is to say they are not in their natural environment but that they have been able to be isolated or obtained by purification from natural sources, or else obtained by genetic recombination, or by chemical synthesis, and that they can then contain unnatural amino acids as will be described further on.

More particularly, according to another embodiment of the disclosure, it is claimed an antibody, or one of its functional fragments or derivatives, said antibody being characterized in that it comprises at least one complementary determining region CDR chosen from CDRs comprising the amino acid sequence SEQ ID Nos. 1 to 12.

More particularly, according to another embodiment of the disclosure, it is claimed an antibody, or one of its functional fragments or derivatives, said antibody being characterized in that it comprises at least one complementary determining region CDR chosen from CDRs comprising the amino acid sequence SEQ ID Nos. 2, 5 or 40 to 49.

According to an embodiment, the disclosure relates to an isolated antibody, or a derived compound or functional fragment of same, comprising at least one CDR chosen among the CDRs of sequences SEQ ID Nos. 1, 2, 3, 4, 5 or 6 or at least one CDR whose sequence has at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID No. 1, 2, 3, 4, 5 or 6.

According to another embodiment, the disclosure relates to an isolated antibody, or a derived compound or functional fragment of same, comprising at least one CDR chosen

among the CDRs of sequences SEQ ID Nos. 40, 2, 41, 42, 5 or 43 or at least one CDR whose sequence has at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID Nos. 40, 2, 41, 42, 5 or 43.

In one aspect, there is provided a process for the selection of an anti-CXCR4 antibody, or one of its functional fragments or derivatives, capable of inhibiting activation of CXCR4, characterized in that it comprises the following steps:

i) screening the generated antibodies and selecting antibodies capable to bind specifically to CXCR4 and also to modulate CXCR4 activation;

ii) testing the selected antibodies of step i) and selecting antibodies capable to induce CXCR4 homodimers conformational change, and then

iii) testing the selected antibodies of step ii) and selecting antibodies capable to induce CXCR4/CXCR2 heterodimers conformational change.

In another aspect, there is provided an isolated antibody, or a derived compound or functional fragment of same, susceptible of being obtained by a process as defined above, said antibody consisting of a monoclonal antibody capable of inhibiting at least 40 %, of the BRET signal on cells expressing both CXCR4-RLuc/CXCR4-YFP and/or CXCR4-RLuc/CXCR2-YFP.

In another aspect, there is provided an antibody, or a derived compound or functional fragment of same, capable of inhibiting activation of CXCR4, characterized in that the said antibody, or derived compound or functional fragment of same, is selected in the group consisting of:

a) an antibody, or a derived compound or functional fragment of same, comprising:

- a light chain comprising, according to IMGT, the CDR-L1 of the sequence SEQ ID No. 1, the CDR-L2 of the sequence SEQ ID No. 2 and the CDR-L3 of the sequence SEQ ID No. 3; and

- a heavy chain comprising, according to IMGT, the CDR-H1 of the sequence SEQ ID No. 7, the CDR-H2 of the sequence SEQ ID No. 5 and the CDR-H3 of the sequence SEQ ID No. 8;

b) an antibody, or a derived compound or functional fragment of same, comprising:

- a light chain comprising, according to Kabat, the CDR-L1 of the sequence SEQ ID No. 9, the CDR-L2 of the sequence SEQ ID No. 10 and the CDR-L3 of the sequence SEQ ID No. 3; and

- a heavy chain comprising, according to Kabat, the CDR-H1 of the sequence SEQ ID No. 11, the CDR-H2 of the sequence SEQ ID No. 12 and the CDR-H3 of the sequence SEQ ID No. 6;

c) an antibody, or a derived compound or functional fragment of same, comprising:

- a light chain comprising, according to IMGT, the CDR-L1 of the sequence SEQ ID No. 40, the CDR-L2 of the sequence SEQ ID No. 2 and the CDR-L3 of the sequence SEQ ID No. 41; and

- a heavy chain comprising, according to IMGT, the CDR-H1 of the sequence SEQ ID No. 44, the CDR-H2 of the sequence SEQ ID No. 5 and the CDR-H3 of the sequence SEQ ID No. 45; and

d) an antibody, or a derived compound or functional fragment of same, comprising:

- a light chain comprising, according to Kabat, the CDR-L1 of the sequence SEQ ID No. 46, the CDR-L2 of the sequence SEQ ID No. 47 and the CDR-L3 of the sequence SEQ ID No. 41; and

- a heavy chain comprising, according to Kabat, the CDR-H1 of the sequence SEQ ID No. 48, the CDR-H2 of the sequence SEQ ID No. 49 and the CDR-H3 of the sequence SEQ ID No. 43.

In another aspect, there is provided a murine hybridoma selected from hybridoma filed at the CNCM, Pasteur Institute, Paris, on October 22, 2007, under number I-3860 or filed at the CNCM, Pasteur Institute, Paris, on June 25, 2008, under number I-4019.

In another aspect, there is provided an antibody secreted by the hybridoma as defined above.

In another aspect, there is provided an isolated nucleic acid characterized in that the said isolated nucleic acid is selected among the following nucleic acids:

- a nucleic acid, DNA or RNA, coding for an antibody, or for a derived compound or functional fragment of same, as defined above;

- a nucleic acid complementary to a nucleic acid as defined in a); and

- a nucleic acid of at least 18 nucleotides capable of hybridizing under highly stringent conditions with at least one of the CDRs of nucleic acid sequences SEQ ID Nos. 15 to 26 or SEQ ID Nos. 52 to 61.

In another aspect, there is provided a vector composed of a nucleic acid as defined above.

In another aspect, there is provided a host cell comprising a vector as defined above.

In another aspect, there is provided a transgenic animal, except for man, comprising a cell transformed by a vector as defined above.

In another aspect, there is provided a method for producing an antibody, or a derived compound or functional fragment of same, as defined above, characterized in that said method comprises the following steps:

- the culture in a medium of and the suitable culture conditions for a host cell as defined above; and

- the recovery of said antibody, or one of its functional fragments, thus produced from the culture medium or from said cultured cells.

In another aspect, there is provided an antibody, or a derived compound or functional fragment of same, as defined above, for use as a drug.

In another aspect, there is provided a pharmaceutical composition comprising as an active ingredient a compound consisting of an antibody, or a derived compound or functional fragment of same, as defined above.

In another aspect, there is provided a pharmaceutical composition as defined above, for use as a drug.

In another aspect, there is provided an antibody, or a derived compound or functional fragment of same, as defined above, and/or of a pharmaceutical composition as defined above, as a drug for modulating CXCR4 activity in a cell.

In another aspect, there is provided the use of an antibody, or a derived compound or functional fragment of same, as defined above, and/or of a pharmaceutical composition as defined above, in the manufacture of a medicament for prevention or treatment of cancer.

A "functional fragment" of an antibody means in particular an antibody fragment, such as fragments Fv, scFv (sc=single chain), Fab, $F(ab')_2$, Fab', scFv-Fc or diabodies, or any fragment whose half-life has been increased. Such functional fragments will be described in detail later in the present description.

A "derived compound" or "derivative" of an antibody means in particular a binding protein composed of a peptide scaffold and at least one of the CDRs of the original antibody in order to preserve its ability to recognize CXCR4. Such derived compounds, well-known to a person skilled in the art, will be described in more detail later in the present description.

More preferably, the invention comprises the antibodies, their derived compounds or their functional fragments, according to the present invention, notably chimeric or humanized, obtained by genetic recombination or chemical synthesis.

According to a preferred embodiment, the antibody according to the invention, or its derived compounds or functional fragments, is characterized in that it consists of a monoclonal antibody.

"Monoclonal antibody" is understood to mean an antibody arising from a nearly homogeneous antibody population. More particularly, the individual antibodies of a population are identical except for a few possible naturally-occurring mutations which can be found in minimal proportions. In other words, a monoclonal antibody consists of a homogeneous antibody arising from the growth of a single cell clone (for example a hybridoma, a eukaryotic host cell transfected with a DNA molecule coding for the homogeneous antibody, a prokaryotic host cell transfected with a DNA molecule coding for the homogeneous antibody, etc.) and is generally characterized by heavy chains of one and only one class and subclass, and light chains of only one type. Monoclonal antibodies are highly specific and are directed against a single antigen. In addition, in contrast with preparations of polyclonal antibodies which typically include various antibodies directed against various determinants, or epitopes, each monoclonal antibody is directed against a single epitope of the antigen.

It must be understood here that the invention does not relate to antibodies in natural form, i.e., they are not taken from their natural environment but are isolated or obtained by purification from natural sources or obtained by genetic recombination or chemical synthesis and thus they can carry unnatural amino acids as will be described below.

More particularly, according to a preferred embodiment of the invention, the antibody, or its derived compounds or functional fragments, is characterized in that it comprises a light chain comprising at least one CDR chosen among the CDRs of amino acid sequences SEQ ID No. 1, 2 or 3, or at least one CDR whose sequence has at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID No. 1, 2 or 3; or it comprises a heavy chain comprising at least one CDR chosen among the CDRs of amino acid sequences SEQ ID Nos. 4, 5 or 6, or at least one CDR whose sequence has at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID Nos. 4, 5 or 6.

According to another embodiment, the antibodies of the invention, or one of their derived compounds or functional fragments, are characterized in that they comprise a light chain comprising at least one of the three CDRs of the sequences SEQ ID No. 1, 2 or 3, or at least one sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID Nos. 1, 2 or 3.

In a preferred manner, the antibodies of the invention, or one of their derived compounds or functional fragments, are characterized in that they comprise a light chain comprising the following three CDRs, respectively CDR-L1, CDR-L2 and CDR-L3, wherein:

- CDR-L1 comprises the sequence SEQ ID No. 1 or 9, or a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 1 or 9;
- CDR-L2 comprises the sequences SEQ ID No. 2 or 10, or a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 2 or 10; and
- CDR-L3 comprises the sequence SEQ ID No. 3, or a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 3.

According to a particular embodiment, antibodies, or one of their derived compounds or functional fragments, are characterized in that they comprise a light chain

comprising the CDR-L1 of the sequence SEQ ID No. 1, the CDR-L2 of the sequence SEQ ID No. 2 and the CDR-L3 of the sequence SEQ ID No. 3.

According to another particular embodiment, antibodies, or one of their derived compounds or functional fragments, are characterized in that they comprise a light chain comprising the CDR-L1 of the sequence SEQ ID No. 9, the CDR-L2 of the sequence SEQ ID No. 10 and the CDR-L3 of the sequence SEQ ID No. 3.

More particularly, the antibodies of the invention, or one of their derived compounds or functional fragments, are characterized in that they comprise a heavy chain comprising at least one of the three CDRs of the sequences SEQ ID Nos. 4, 5 or 6, or at least one sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID Nos. 4, 5 or 6.

Even more preferably, the antibodies of the invention, or one of their derived compounds or functional fragments, are characterized in that they comprise a heavy chain comprising the following three CDRs, respectively CDR-H1, CDR-H2 and CDR-H3, wherein:

- CDR-H1 comprises the sequence SEQ ID Nos. 4, 7 or 11, or a sequence with at least 80% identity after optimal alignment with sequence SEQ ID Nos. 4, 7 or 11;
- CDR-H2 comprises the sequences SEQ ID Nos. 5 or 12, or a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 5 or 12; and
- CDR-H3 comprises the sequences SEQ ID No. 6 or 8, or a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 6 or 8.

According to a particular embodiment, antibodies, or one of their derived compounds or functional fragments, are characterized in that they comprise a heavy chain comprising the CDR-H1 of the sequence SEQ ID No. 7, the CDR-H2 of the sequence SEQ ID No. 5 and the CDR-H3 of the sequence SEQ ID No. 8.

According to another particular embodiment, antibodies, or one of their derived compounds or functional fragments, are characterized in that they comprise a heavy chain comprising the CDR-H1 of the sequence SEQ ID No. 11, the CDR-H2 of the sequence SEQ ID No. 12 and the CDR-H3 of the sequence SEQ ID No. 6.

More particularly, according to a preferred embodiment of the invention, the antibody, or its derived compounds or functional fragments, is characterized in that it comprises a light chain comprising at least one CDR chosen among the CDRs of amino

acid sequences SEQ ID Nos. 40, 2 or 41 or at least one CDR whose sequence has at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID No. 40, 2 or 41; or it comprises a heavy chain comprising at least one CDR chosen among the CDRs of amino acid sequences SEQ ID Nos. 42, 5 or 43, or at least one CDR whose sequence has at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID Nos. 42, 5 or 43.

According to another embodiment, the antibodies of the invention, or one of their derived compounds or functional fragments, are characterized in that they comprise a light chain comprising at least one of the three CDRs of the sequences SEQ ID Nos. 40, 2 or 41, or at least one sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID Nos. 40, 2 or 41.

In a preferred manner, the antibodies of the invention, or one of their derived compounds or functional fragments, are characterized in that they comprise a light chain comprising the following three CDRs, respectively CDR-L1, CDR-L2 and CDR-L3, wherein:

- CDR-L1 comprises the sequence SEQ ID No. 40 or 46, or a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 40 or 46;
- CDR-L2 comprises the sequences SEQ ID No. 2 or 47, or a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 2 or 47; and
- CDR-L3 comprises the sequence SEQ ID No. 4 or a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No 41.

According to a particular embodiment, antibodies, or one of their derived compounds or functional fragments, are characterized in that they comprise a light chain comprising the CDR-L1 of the sequence SEQ ID No. 40, the CDR-L2 of the sequence SEQ ID No. 2 and the CDR-L3 of the sequence SEQ ID No. 41.

According to another particular embodiment, antibodies, or one of their derived compounds or functional fragments, are characterized in that they comprise a light chain comprising the CDR-L1 of the sequence SEQ ID No. 46, the CDR-L2 of the sequence SEQ ID No. 47 and the CDR-L3 of the sequence SEQ ID No. 41.

More particularly, the antibodies of the invention, or one of their derived compounds or functional fragments, are characterized in that they comprise a heavy chain comprising at least one of the three CDRs of the sequences SEQ ID Nos. 42, 5 or

43, or at least one sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID Nos. 42, 5 or 43.

Even more preferably, the antibodies of the invention, or one of their derived compounds or functional fragments, are characterized in that they comprise a heavy chain comprising the following three CDRs, respectively CDR-H1, CDR-H2 and CDR-H3, wherein:

- CDR-H1 comprises the sequence SEQ ID Nos. 42, 44 or 48, or a sequence with at least 80% identity after optimal alignment with sequence SEQ ID Nos. 42, 44 or 48;
- CDR-H2 comprises the sequences SEQ ID No. 5 or 49, or a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 5 or 49; and
- CDR-H3 comprises the sequences SEQ ID No. 45 or 43, or a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 45 or 43.

According to a particular embodiment, antibodies, or one of their derived compounds or functional fragments, are characterized in that they comprise a heavy chain comprising the CDR-H1 of the sequence SEQ ID No. 44, the CDR-H2 of the sequence SEQ ID No. 5 and the CDR-H3 of the sequence SEQ ID No. 45.

According to another particular embodiment, antibodies, or one of their derived compounds or functional fragments, are characterized in that they comprise a heavy chain comprising the CDR-H1 of the sequence SEQ ID No. 48, the CDR-H2 of the sequence SEQ ID No. 49 and the CDR-H3 of the sequence SEQ ID No. 43.

In the present description, the terms "polypeptides", "polypeptide sequences", "peptides" and "proteins attached to antibody compounds or to their sequences" are interchangeable.

It must be understood here that the invention does not relate to antibodies in natural form, i.e., they are not taken from their natural environment but are isolated or obtained by purification from natural sources or obtained by genetic recombination or chemical synthesis and thus they can carry unnatural amino acids as will be described below.

In a first embodiment, complementarity-determining region, or CDR, means the hypervariable regions of the heavy and light chains of immunoglobulins as defined by Kabat *et al.* (Kabat *et al.*, Sequences of proteins of immunological interest, 5th Ed., U.S. Department of Health and Human Services, NIH, 1991, and later editions). There are

three heavy-chain CDRs and three light-chain CDRs. Here, the terms "CDR" and "CDRs" are used to indicate, depending on the case, one or more, or even all, of the regions containing the majority of the amino acid residues responsible for the antibody's binding affinity for the antigen or epitope it recognizes.

In a second embodiment, by CDR regions or CDR(s), it is intended to indicate the hypervariable regions of the heavy and light chains of the immunoglobulins as defined by IMGT.

The IMGT unique numbering has been defined to compare the variable domains whatever the antigen receptor, the chain type, or the species [Lefranc M.-P., Immunology Today 18, 509 (1997) / Lefranc M.-P., The Immunologist, 7, 132-136 (1999) / Lefranc, M.-P., Pommié, C., Ruiz, M., Giudicelli, V., Foulquier, E., Truong, L., Thouvenin-Contet, V. and Lefranc, Dev. Comp. Immunol., 27, 55-77 (2003)]. In the IMGT unique numbering, the conserved amino acids always have the same position, for instance cystein 23 (1st-CYS), tryptophan 41 (CONSERVED-TRP), hydrophobic amino acid 89, cystein 104 (2nd-CYS), phenylalanine or tryptophan 118 (J-PHE or J-TRP). The IMGT unique numbering provides a standardized delimitation of the framework regions (FR1-IMGT: positions 1 to 26, FR2-IMGT: 39 to 55, FR3-IMGT: 66 to 104 and FR4-IMGT: 118 to 128) and of the complementarity determining regions: CDR1-IMGT: 27 to 38, CDR2-IMGT: 56 to 65 and CDR3-IMGT: 105 to 117. As gaps represent unoccupied positions, the CDR-IMGT lengths (shown between brackets and separated by dots, e.g. [8.8.13]) become crucial information. The IMGT unique numbering is used in 2D graphical representations, designated as IMGT Colliers de Perles [Ruiz, M. and Lefranc, M.-P., Immunogenetics, 53, 857-883 (2002) / Kaas, Q. and Lefranc, M.-P., Current Bioinformatics, 2, 21-30 (2007)], and in 3D structures in IMGT/3Dstructure-DB [Kaas, Q., Ruiz, M. and Lefranc, M.-P., T cell receptor and MHC structural data. Nucl. Acids. Res., 32, D208-D210 (2004)].

Three heavy chain CDRs and 3 light chain CDRs exist. The term CDR or CDRs is used here in order to indicate, according to the case, one of these regions or several, or even the whole, of these regions which contain the majority of the amino acid residues responsible for the binding by affinity of the antibody for the antigen or the epitope which it recognizes.

For more clarity, it must be understood that in the following description, and more particularly in tables 2 and 3, the CDRs will be defined by IMGT numbering, Kabat numbering and by common numbering.

Common numbering regroups the residues part of each CDR which are common to the CDRs as defined by the IMGT and the Kabat numbering systems.

IMGT numbering system defines the CDRs according to the IMGT system as above defined whereas Kabat numbering system defines the CDRs according to the Kabat system as above defined.

More particularly, CDR-L1 consists of SEQ ID No. 1 (**QSLYNSRTRKNY**) in the common and IMGT numbering systems and of SEQ ID No. 9 (KSS**QSLYNSRTRKNY**LA) in the Kabat numbering system.

Concerning the CDR-L2, it consists of SEQ ID No. 2 (**WAS**) in the common and IMGT numbering systems and of SEQ ID No. 10 (**WAS**TRES) in the Kabat numbering system.

The CDR-L3 consists of SEQ ID No. 3 (**KQSYNLRT**) for each of the three numbering systems.

For the heavy chain, the CDR-H1 consists of the SEQ ID No. 4 (**TDYY**) in the common numbering system, of SEQ ID No. 7 (GFTF**TDYY**) in the IMGT numbering system and of SEQ ID No. 11 (**TDYY**MS) in the kabat numbering system.

The CDR-H2 consists of SEQ ID No. 5 (**IRNKANGYTT**) in the common and IMGT numbering systems and of SEQ ID No. 12 (**FIRNKANGYTT**EYSASVKG) in the kabat numbering system.

At last, the CDR-H3 consists in the SEQ ID No. 6 (**DIPGFAY**) in the common and kabat numbering systems whereas it consists of SEQ ID No. 8 (AR**DIPGFAY**) in the IMGT numbering system.

More particularly, CDR-L1 consists of SEQ ID No. 40 (**QSLFNSRTRKNY**) in the common and IMGT numbering systems and of SEQ ID No. 46 (KSS**QSLFNSRTRKNY**LA) in the Kabat numbering system.

Concerning the CDR-L2, it consists of SEQ ID No. 2 (**WAS**) in the common and IMGT numbering systems and of SEQ ID No. 47 (**WAS**ARDS) in the Kabat numbering system.

The CDR-L3 consists of SEQ ID No. 41 (**MQSFNLRT**) for each of the three numbering systems.

For the heavy chain, the CDR-H1 consists of the SEQ ID No. 42 (**DNY**) in the common numbering system, of SEQ ID No. 44 (GFTF**TDNY**) in the IMGT numbering system and of SEQ ID No. 48 (**DNY**MS) in the kabat numbering system.

The CDR-H2 consists of SEQ ID No. 5 (**IRNKANGYTT**) in the common and IMGT numbering systems and of SEQ ID No. 49 (**FIRNKANGYTT**DYSASVRG) in the kabat numbering system.

At last, the CDR-H3 consists in the SEQ ID No. 43 (**DVGSNYFDY**) in the common and kabat numbering systems whereas it consists of SEQ ID No. 45 (AR**DVGSNYFDY**) in the IMGT numbering system.

In the sense of the present invention, the "percentage identity" between two sequences of nucleic acids or amino acids means the percentage of identical nucleotides or amino acid residues between the two sequences to be compared, obtained after optimal alignment, this percentage being purely statistical and the differences between the two sequences being distributed randomly along their length. The comparison of two nucleic acid or amino acid sequences is traditionally carried out by comparing the sequences after having optimally aligned them, said comparison being able to be conducted by segment or by using an "alignment window". Optimal alignment of the sequences for comparison can be carried out, in addition to comparison by hand, by means of the local homology algorithm of Smith and Waterman (1981) [Ad. App. Math. 2:482], by means of the local homology algorithm of Neddleman and Wunsch (1970) [J. Mol. Biol. 48:443], by means of the similarity search method of Pearson and Lipman (1988) [Proc. Natl. Acad. Sci. USA 85:2444] or by means of computer software using these algorithms (GAP, BESTFIT, FASTA and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI, or by the comparison software BLAST NR or BLAST P).

The percentage identity between two nucleic acid or amino acid sequences is determined by comparing the two optimally-aligned sequences in which the nucleic acid or amino acid sequence to compare can have additions or deletions compared to the reference sequence for optimal alignment between the two sequences. Percentage identity is calculated by determining the number of positions at which the amino acid

nucleotide or residue is identical between the two sequences, preferably between the two complete sequences, dividing the number of identical positions by the total number of positions in the alignment window and multiplying the result by 100 to obtain the percentage identity between the two sequences.

For example, the BLAST program, "BLAST 2 sequences" (Tatusova et al., "Blast 2 sequences - a new tool for comparing protein and nucleotide sequences", FEMS Microbiol., 1999, Lett. 174:247–250) available on the site http://www.ncbi.nlm.nih.gov/gorf/bl2.html, can be used with the default parameters (notably for the parameters "open gap penalty": 5, and "extension gap penalty": 2; the selected matrix being for example the "BLOSUM 62" matrix proposed by the program); the percentage identity between the two sequences to compare is calculated directly by the program.

For the amino acid sequence exhibiting at least 80%, preferably 85%, 90%, 95% and 98% identity with a reference amino acid sequence, preferred examples include those containing the reference sequence, certain modifications, notably a deletion, addition or substitution of at least one amino acid, truncation or extension. In the case of substitution of one or more consecutive or non-consecutive amino acids, substitutions are preferred in which the substituted amino acids are replaced by "equivalent" amino acids. Here, the expression "equivalent amino acids" is meant to indicate any amino acids likely to be substituted for one of the structural amino acids without however modifying the biological activities of the corresponding antibodies and of those specific examples defined below.

Equivalent amino acids can be determined either on their structural homology with the amino acids for which they are substituted or on the results of comparative tests of biological activity between the various antibodies likely to be generated.

As a non-limiting example, table 1 below summarizes the possible substitutions likely to be carried out without resulting in a significant modification of the biological activity of the corresponding modified antibody; inverse substitutions are naturally possible under the same conditions.

Table 1

Original residue	Substitution(s)
Ala (A)	Val, Gly, Pro
Arg (R)	Lys, His
Asn (N)	Gln
Asp (D)	Glu
Cys (C)	Ser
Gln (Q)	Asn
Glu (G)	Asp
Gly (G)	Ala
His (H)	Arg
Ile (I)	Leu
Leu (L)	Ile, Val, Met
Lys (K)	Arg
Met (M)	Leu
Phe (F)	Tyr
Pro (P)	Ala
Ser (S)	Thr, Cys
Thr (T)	Ser
Trp (W)	Tyr
Tyr (Y)	Phe, Trp
Val (V)	Leu, Ala

It is known by those skilled in the art that in the current state of the art the greatest variability (length and composition) between the six CDRs is found at the three heavy-chain CDRs and, more particularly, at CDR-H3 of this heavy chain. Consequently, it will be evident that the preferred characteristic CDRs of the antibodies of the invention, or of one of their derived compounds or functional fragments, will be the three CDRs of the heavy chain, i.e., for the 414H5 the CDRs coded by sequences SEQ ID Nos. 7, 5, 8 and 11, 12, 6, respectively defined according to IMGT and Kabat and, for the 515H7, the CDRs coded by sequences SEQ ID Nos. 44, 5, 45 and 48, 49, 43, respectively defined according to IMGT and Kabat. Even more preferentially, the CDR corresponding to the CDR-H3 coded by sequence SEQ ID No. 8 or 6 for the 414H5 and 45 or 43 for the 515H7.

In a specific embodiment, the present invention relates to a murine antibody, or derived compounds or functional fragments of same.

Another embodiment of the invention discloses an antibody, or its derived compounds or functional fragments, comprising a light chain comprising the following three CDRs:

CDR-L1 of the sequence SEQ ID No. 1 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 1;

CDR-L2 of the sequence SEQ ID No. 2 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 2; and

CDR-L3 of the sequence SEQ ID No. 3 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 3, and

a heavy chain comprising the following three CDRs:

CDR-H1 of the sequence SEQ ID No. 4 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 4;

CDR-H2 of the sequence SEQ ID No. 5 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 5; and

CDR-H3 of the sequence SEQ ID No. 6 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 6.

Still another embodiment of the invention discloses an antibody, or a derived compound or functional fragment of same, comprising a light chain comprising the following three CDRs:

- CDR-L1 of the sequence SEQ ID No. 1 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 1;
- CDR-L2 of the sequence SEQ ID No. 2 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 2; and
- CDR-L3 of the sequence SEQ ID No. 3 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 3, and

a heavy chain comprising the following three CDRs:

- CDR-H1 of the sequence SEQ ID No. 7 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 7;

- CDR-H2 of the sequence SEQ ID No. 5 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 5; and
- CDR-H3 of the sequence SEQ ID No. 8 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 8.

Still another embodiment of the invention discloses an antibody, or a derived compound or functional fragment of same, comprising a light chain comprising the following three CDRs:
- CDR-L1 of the sequence SEQ ID No. 9 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 9;
- CDR-L2 of the sequence SEQ ID No. 10 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 10; and
- CDR-L3 of the sequence SEQ ID No. 3 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 3, and

a heavy chain comprising the following three CDRs:
- CDR-H1 of the sequence SEQ ID No. 11 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 11;
- CDR-H2 of the sequence SEQ ID No. 12 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 12; and
- CDR-H3 of the sequence SEQ ID No. 6 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 6.

An antibody, or a derived compound or functional fragment of same, according to the invention is characterized in that it comprises:

- a light chain comprising the CDR-L1 of the sequence SEQ ID No. 1, the CDR-L2 of the sequence SEQ ID No. 2 and the CDR-L3 of the sequence SEQ ID No. 3; and

- a heavy chain comprising the CDR-H1 of the sequence SEQ ID No. 7, the CDR-H2 of the sequence SEQ ID No. 5 and the CDR-H3 of the sequence SEQ ID No. 8.

In another embodiment, an antibody, or a derived compound or functional fragment of same, according to the invention is characterized in that it comprises:

- a light chain comprising the CDR-L1 of the sequence SEQ ID No. 9, the CDR-L2 of the sequence SEQ ID No. 10 and the CDR-L3 of the sequence SEQ ID No. 3; and

- a heavy chain comprising the CDR-H1 of the sequence SEQ ID No. 11, the CDR-H2 of the sequence SEQ ID No. 12 and the CDR-H3 of the sequence SEQ ID No. 6.

According to still another embodiment, the antibody of the invention, or its derived compounds or functional fragments, is characterized in that it comprises a light-chain sequence comprising the amino acid sequence SEQ ID No. 13 or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 13; and in that it comprises a heavy-chain sequence comprising the amino acid sequence SEQ ID No. 14 or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 14.

Another embodiment of the invention discloses an antibody, or its derived compounds or functional fragments, comprising a light chain comprising the following three CDRs:

CDR-L1 of the sequence SEQ ID No. 40 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 40;

CDR-L2 of the sequence SEQ ID No. 2 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 2; and

CDR-L3 of the sequence SEQ ID No. 41 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 41, and

a heavy chain comprising the following three CDRs:

CDR-H1 of the sequence SEQ ID No. 42 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 42;

CDR-H2 of the sequence SEQ ID No. 5 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 5; and

CDR-H3 of the sequence SEQ ID No. 43 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 43.

Still another embodiment of the invention discloses an antibody, or a derived compound or functional fragment of same, comprising a light chain comprising the following three CDRs:

- CDR-L1 of the sequence SEQ ID No. 40 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 40;
- CDR-L2 of the sequence SEQ ID No. 2 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 2; and
- CDR-L3 of the sequence SEQ ID No. 41 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 41, and

a heavy chain comprising the following three CDRs:

- CDR-H1 of the sequence SEQ ID No. 44 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 44;
- CDR-H2 of the sequence SEQ ID No. 5 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 5; and
- CDR-H3 of the sequence SEQ ID No. 45 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 45.

Still another embodiment of the invention discloses an antibody, or a derived compound or functional fragment of same, comprising a light chain comprising the following three CDRs:

- CDR-L1 of the sequence SEQ ID No. 46 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 46;
- CDR-L2 of the sequence SEQ ID No. 47 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 47; and
- CDR-L3 of the sequence SEQ ID No. 41 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 41, and

a heavy chain comprising the following three CDRs:

- CDR-H1 of the sequence SEQ ID No. 48 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 48;
- CDR-H2 of the sequence SEQ ID No. 49 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 49; and
- CDR-H3 of the sequence SEQ ID No. 43 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 43.

An antibody, or a derived compound or functional fragment of same, according to the invention is characterized in that it comprises:

- a light chain comprising the CDR-L1 of the sequence SEQ ID No. 40, the CDR-L2 of the sequence SEQ ID No. 2 and the CDR-L3 of the sequence SEQ ID No. 41; and
- a heavy chain comprising the CDR-H1 of the sequence SEQ ID No. 44, the CDR-H2 of the sequence SEQ ID No. 5 and the CDR-H3 of the sequence SEQ ID No. 45.

In another embodiment, an antibody, or a derived compound or functional fragment of same, according to the invention is characterized in that it comprises:

- a light chain comprising the CDR-L1 of the sequence SEQ ID No. 46, the CDR-L2 of the sequence SEQ ID No. 47 and the CDR-L3 of the sequence SEQ ID No. 41; and
- a heavy chain comprising the CDR-H1 of the sequence SEQ ID No. 48, the CDR-H2 of the sequence SEQ ID No. 49 and the CDR-H3 of the sequence SEQ ID No. 43.

According to still another embodiment, the antibody of the invention, or its derived compounds or functional fragments, is characterized in that it comprises a light-chain sequence comprising the amino acid sequence SEQ ID No. 50 or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 50; and in that it comprises a heavy-chain sequence comprising the amino acid sequence SEQ ID No. 51 or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 51.

As seen above, the invention also relates to any compound derived from an antibody as described in the invention.

More particularly, the antibody of the invention, or its derived compounds or functional fragments, is characterized in that said derived compound consists of a binding protein comprising a peptide scaffold on which is grafted at least one CDR in such a way as to preserve all or part of the paratope recognition properties of the initial antibody.

One or more sequences among the six CDR sequences described in the present invention can also be present on the various immunoglobulin protein scaffolding. In this case, the protein sequence makes it possible to recreate a peptide skeleton favorable to the folding of the grafted CDRs, enabling them to preserve their paratope antigen-recognition properties.

Generally, a person skilled in the art knows how to determine the type of protein scaffold on which to graft at least one of the CDRs arising from the original antibody. More particularly, it is known that to be selected such scaffolds must meet the greatest number of criteria as follows (Skerra A., J. Mol. Recogn., 2000, 13:167-187):

- good phylogenetic conservation;
- known three-dimensional structure (as, for example, by crystallography, NMR spectroscopy or any other technique known to a person skilled in the art);
- small size;
- few or no post-transcriptional modifications; and/or
- easy to produce, express and purify.

The origin of such protein scaffolds can be, but is not limited to, the structures selected among: fibronectin and preferentially fibronectin type III domain 10, lipocalin, anticalin (Skerra A., J. Biotechnol., 2001, 74(4):257–75), protein Z arising from domain B of protein A of *Staphylococcus aureus*, thioredoxin A or proteins with a repeated motif such as the "ankyrin repeat" (Kohl *et al.*, PNAS, 2003, vol. 100, No. 4, 1700-1705), the "armadillo repeat", the "leucine-rich repeat" and the "tetratricopeptide repeat".

Scaffolds derived from toxins such as, for example, toxins from scorpions, insects, plants, mollusks, etc., and the protein inhibiters of neuronal NO synthase (PIN) should also be mentioned.

An example, in no way limiting, of such hybrid constructions, is the insertion of the CDR-H1 (heavy chain) of an antiCD4 antibody, namely 13B8.2, in one of the loops in the PIN, the new binding protein thus obtained preserving the same binding properties as the original antibody (Bes *et al.*, Biochem. Biophys. Res. Commun., 2006, 343(1), 334-344). On a purely illustrative basis, grafting the CDR-H3 (heavy chain) of an anti-lysozyme VHH antibody on one of the loops of neocarzinostatin (Nicaise *et al.*, Protein Science, 2004, 13(7):1882-1891) can also be mentioned.

Lastly, as described above, such peptide scaffolds can comprise from one to six CDRs arising from the original antibody. Preferably, but not being a requirement, a person skilled in the art will select at least one CDR from the heavy chain, the latter being known to be primarily responsible for the specificity of the antibody. The selection of one or more relevant CDRs is obvious to a person skilled in the art, who will then choose suitable known techniques (Bes *et al.*, FEBS letters 508, 2001, 67-74).

A specific aspect of the present invention relates to a method for selecting a compound derived from an antibody according to the invention, said derived compound being capable of inhibiting *in vitro* and/or *in vivo* the growth of tumor cells and said derived compound comprising a peptide scaffold on which is grafted at least one antibody CDR, characterized in that it comprises the following steps:

a) the placing in contact *in vitro* of a compound composed of a peptide scaffold on which is grafted at least one antibody CDR with a biological sample containing tumor cells able to grow and under conditions allowing these cells to grow; and

b) selection of said compound if said compound is capable of inhibiting the growth of these tumor cells,

and characterized in that said at least one grafted CDR is selected among the following CDRs:

- the CDR of sequence SEQ ID No. 1, 9, 40, 46 or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 1, 9, 40, 46;
- the CDR of sequence SEQ ID No. 2, 10, 47 or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 2, 10, 47;

- the CDR of sequence SEQ ID No. 3, 41 or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 3, 41;
- the CDR of sequence SEQ ID No. 4, 7, 11, 42, 44, 48 or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 4, 7, 11, 42, 44, 48;
- the CDR of sequence SEQ ID No. 5, 12, 49 or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 5, 12, 49; and
- the CDR of sequence SEQ ID No. 6, 8, 43, 45 or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 6, 8, 43, 45.

According to a preferred mode, the method can include in step a) the placing in contact *in vitro* of a compound comprising a peptide scaffold on which is grafted at least two or three antibody CDRs.

According to an even more preferred mode of this method, the peptide scaffold is selected among the scaffolds or binding proteins whose structures were mentioned above.

Obviously, these examples are in no way limiting, and any other structure known or obvious to a person skilled in the art should be considered as being covered by the protection conferred by the present patent application.

The present invention thus relates to an antibody, or its derived compounds or functional fragments, characterized in that the peptide scaffold is selected among proteins that are a) phylogenetically well preserved, b) of robust architecture, c) with a well-known 3-D molecular organization, d) of small size and/or e) comprising regions that can be modified by deletion and/or insertion without modifying stability properties.

According to a preferred embodiment, the antibody of the invention, or its derived compounds or functional fragments, is characterized in that said peptide scaffold is selected among i) scaffolds arising from fibronectin, preferentially fibronectin type 3 domain 10, lipocalin, anticalin, protein Z arising from domain B of protein A of *Staphylococcus aureus*, thioredoxin A or proteins with a repeated motif such as the "ankyrin repeat" (Kohl *et al.*, PNAS, 2003, vol. 100, No. 4, 1700–1705), the

"armadillo repeat", the "leucine-rich repeat" and the "tetratricopeptide repeat" or iii) protein inhibiters of neuronal NO synthase (PIN).

Another aspect of the invention relates to the functional fragments of the antibody described above.

More particularly, the invention targets an antibody, or its derived compounds or functional fragments, characterized in that said functional fragment is selected among the fragments Fv, Fab, $(Fab')_2$, Fab', scFv, scFv-Fc and diabodies, or any fragment whose half–life has been increased such as PEGylated fragments.

Such functional fragments of the antibody according to the invention consist, for example, of the fragments Fv, scFv (sc=simple chain), Fab, $F(ab')_2$, Fab', scFv-Fc or diabodies, or any fragment whose half-life has been increased by chemical modification, such as the addition of polyalkylene glycol such as polyethylene glycol (PEGylation) (PEGylated fragments are referred to as Fv-PEG, scFv-PEG, Fab-PEG, $F(ab')_2$-PEG and Fab'-PEG), or by incorporation in a liposome, microspheres or PLGA, said fragments possessing at least one of the characteristic CDRs of the invention which is notably capable of exerting in a general manner activity, even partial, of the antibody from which it arises.

Preferably, said functional fragments will comprise or include a partial sequence of the variable heavy or light chain of the antibody from which they are derived, said partial sequence being sufficient to retain the same binding specificity as the antibody from which it arises and sufficient affinity, preferably at least equal to 1/100, more preferably at least 1/10 of that of the antibody from which it arises.

Such a functional fragment will contain at least five amino acids, preferably 6, 7, 8, 10, 15, 25, 50 or 100 consecutive amino acids of the sequence of the antibody from which it arises.

Preferably, these functional fragments will be of the types Fv, scFv, Fab, $F(ab')_2$, F(ab'), scFv-Fc or diabodies, which generally have the same binding specificity as the antibody from which they result. According to the present invention, fragments of the antibody of the invention can be obtained from the antibodies described above by methods such as enzyme digestion, including pepsin or papain, and/or by cleavage of the disulfide bridges by chemical reduction. The antibody fragments can be also obtained by recombinant genetics techniques also known to a person skilled in the art or

by peptide synthesis by means, for example, of automatic peptide synthesizers such as those sold by Applied BioSystems, etc.

For more clarity, table 2 below summarizes the various amino acid sequences corresponding to the antibody of the invention.

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Table 2 (wherein Mu. = murine and Ch. = chimeric)

Antibody	CDR numbering	Heavy chain	Light chain	SEQ ID NO.
414H5	Common		CDR-L1	1
			CDR-L2	2
			CDR-L3	3
		CDR-H1		4
		CDR-H2		5
		CDR-H3		6
	IMGT		CDR-L1	1
			CDR-L2	2
			CDR-L3	3
		CDR-H1		7
		CDR-H2		5
		CDR-H3		8
	Kabat		CDR-L1	9
			CDR-L2	10
			CDR-L3	3
		CDR-H1		11
		CDR-H2		12
		CDR-H3		6
			Mu. variable domain	13
		Mu. variable domain		14
			Ch. variable domain	64
		Ch. variable domain		65
515H7	Common		CDR-L1	40
			CDR-L2	2
			CDR-L3	41
		CDR-H1		42
		CDR-H2		5
		CDR-H3		43
	IMGT		CDR-L1	40
			CDR-L2	2
			CDR-L3	41
		CDR-H1		44
		CDR-H2		5
		CDR-H3		45
	Kabat		CDR-L1	46
			CDR-L2	47
			CDR-L3	41
		CDR-H1		48
		CDR-H2		49
		CDR-H3		43
			Mu. variable domain	50
		Mu. variable domain		51
			Ch. variable domain	66
		Ch. variable domain		67

Another specific aspect of the present invention relates to a chimeric antibody, or its derived compounds or functional fragments, characterized in that said antibody also comprises light-chain and heavy-chain constant regions derived from an antibody of a species heterologous with the mouse, notably man.

Yet another specific aspect of the present invention relates to a humanized antibody, or its derived compounds or functional fragments, characterized in that the constant regions of the light-chain and the heavy-chain derived from human antibody are, respectively, the lambda or kappa region and the gamma-1, gamma-2 or gamma-4 region.

According to another aspect, the invention relates to a murine hybridoma capable of secreting a monoclonal antibody according to the invention, notably the hybridoma of murine origin filed with the French collection for microorganism cultures (CNCM, Pasteur Institute, Paris, France) on October 22, 2007, under number I-3860. Said hybridoma was obtained by the fusion of Balb/C immunized mice splenocytes and cells of the myeloma Sp 2/O-Ag 14 lines.

The monoclonal antibody, here referred to as 414H5, or its derived compounds or functional fragments, characterized in that said antibody is secreted by the hybridoma filed with the CNCM on October 22, 2007, under number I-3860 obviously forms part of the present invention.

According to another aspect, the invention relates to a murine hybridoma capable of secreting a monoclonal antibody according to the invention, notably the hybridoma of murine origin filed with the French collection for microorganism cultures (CNCM, Pasteur Institute, Paris, France) on June 25, 2008, under number I-4019. Said hybridoma was obtained by the fusion of Balb/C immunized mice splenocytes and cells of the myeloma Sp 2/O-Ag 14 lines.

The monoclonal antibody, here referred to as 515H7, or its derived compounds or functional fragments, characterized in that said antibody is secreted by the hybridoma filed with the CNCM on June 25, 2008, under number I-4019 obviously forms part of the present invention.

The antibody of the invention also comprises chimeric or humanized antibodies.

A chimeric antibody is one containing a natural variable region (light chain and heavy chain) derived from an antibody of a given species in combination with constant

regions of the light chain and the heavy chain of an antibody of a species heterologous to said given species.

The antibodies, or chimeric fragments of same, can be prepared by using the techniques of recombinant genetics. For example, the chimeric antibody could be produced by cloning recombinant DNA containing a promoter and a sequence coding for the variable region of a nonhuman monoclonal antibody of the invention, notably murine, and a sequence coding for the human antibody constant region. A chimeric antibody according to the invention coded by one such recombinant gene could be, for example, a mouse-human chimera, the specificity of this antibody being determined by the variable region derived from the murine DNA and its isotype determined by the constant region derived from human DNA. Refer to Verhoeyn *et al.* (BioEssays, 8:74, 1988) for methods for preparing chimeric antibodies.

In another aspect, the invention describes an antibody, or a derived compound or functional fragment of same, which consists in a chimeric antibody.

In a particular preferred embodiment, the chimeric antibody, or a derived compound or functional fragment of same, of the invention comprises a light chain sequence comprising the amino acid sequence SEQ ID No. 64, and in that it comprises a heavy chain sequence comprising the amino acid sequence SEQ ID No. 65.

In another preferred embodiment, the chimeric antibody, or a derived compound or functional fragment of same, of the invention comprises a light chain sequence comprising the amino acid sequence SEQ ID No. 66, and in that it comprises a heavy chain sequence comprising the amino acid sequence SEQ ID No. 67.

"Humanized antibodies" means an antibody that contains CDR regions derived from an antibody of nonhuman origin, the other parts of the antibody molecule being derived from one (or several) human antibodies. In addition, some of the skeleton segment residues (called FR) can be modified to preserve binding affinity (Jones *et al.*, Nature, 321:522-525, 1986; Verhoeyen *et al.*, Science, 239:1534-1536, 1988; Riechmann *et al.*, Nature, 332:323-327, 1988).

The humanized antibodies of the invention or fragments of same can be prepared by techniques known to a person skilled in the art (such as, for example, those described in the documents Singer *et al.*, J. Immun., 150:2844-2857, 1992; Mountain *et al.*, Biotechnol. Genet. Eng. Rev., 10:1-142, 1992; and Bebbington *et al.*, Bio/Technology,

10:169-175, 1992). Such humanized antibodies are preferred for their use in methods involving *in vitro* diagnoses or preventive and/or therapeutic treatment *in vivo*. Other humanization techniques, also known to a person skilled in the art, such as, for example, the "CDR grafting" technique described by PDL in patents EP 0 451 261, EP 0 682 040, EP 0 939 127, EP 0 566 647 or US 5,530,101, US 6,180,370, US 5,585,089 and US 5,693,761. US patents 5,639,641 or 6,054,297, 5,886,152 and 5,877,293 can also be cited.

In addition, the invention also relates to humanized antibodies arising from the murine antibodies described above.

In a preferred manner, constant regions of the light-chain and the heavy-chain derived from human antibody are, respectively, the lambda or kappa and the gamma-1, gamma-2 or gamma-4 region.

In the embodiment corresponding to IgG1 isotype IgG1, an additional characteristic of the antibody is to exhibit effector functions, such as antibody-dependant cellular cytotoxicity (ADCC) and/or complement–dependant cytotoxicity (CDC).

A novel aspect of the present invention relates to an isolated nucleic acid characterized in that it is selected among the following nucleic acids (including any degenerate genetic code):

- a nucleic acid, DNA or RNA, coding for an antibody, or for a derived compound or functional fragment of same, according to the invention;

- a nucleic acid complementary to a nucleic acid as defined in a);

- a nucleic acid of at least 18 nucleotides capable of hybridizing under highly stringent conditions with at least one of the CDRs of nucleic acid sequences SEQ ID Nos. 15 to 26 or SEQ ID Nos. 52 to 61or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID Nos. 15 to 26 or SEQ ID Nos. 52 to 61; and

- a nucleic acid of at least 18 nucleotides capable of hybridizing under highly stringent conditions with at least the light chain of nucleic acid sequence SEQ ID No. 27 or SEQ ID No. 62 or SEQ ID No. 68 or 70 and/or the heavy chain of nucleic acid sequence SEQ ID No. 28 or SEQ ID No. 63 or SEQ ID No. 69 or 71 or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with

sequences SEQ ID Nos. 27 and/or 28 or SEQ ID Nos. 62 and/or 63 or SEQ ID Nos. 68 and/or 69 or SEQ ID Nos. 70 and/or 71.

Table 3 below summarizes the various nucleotide sequences concerning the antibody of the invention.

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Table 3

Antibody	CDR numbering	Heavy chain	Light chain	SEQ ID NO.
414H5	Common		CDR-L1	15
			CDR-L2	16
			CDR-L3	17
		CDR-H1		18
		CDR-H2		19
		CDR-H3		20
	IMGT		CDR-L1	15
			CDR-L2	16
			CDR-L3	17
		CDR-H1		21
		CDR-H2		19
		CDR-H3		22
	Kabat		CDR-L1	23
			CDR-L2	24
			CDR-L3	17
		CDR-H1		25
		CDR-H2		26
		CDR-H3		20
			Mu. variable domain	27
		Mu. variable domain		28
			Ch. variable domain	68
		Ch. variable domain		69
515H7	Common		CDR-L1	52
			CDR-L2	16
			CDR-L3	53
		CDR-H1		54
		CDR-H2		19
		CDR-H3		55
	IMGT		CDR-L1	52
			CDR-L2	16
			CDR-L3	53
		CDR-H1		56
		CDR-H2		19
		CDR-H3		57
	Kabat		CDR-L1	58
			CDR-L2	59
			CDR-L3	53
		CDR-H1		60
		CDR-H2		61
		CDR-H3		55
			Mu. variable domain	62
		Mu. variable domain		63
			Ch. variable domain	70
		Ch. variable domain		71

The terms "nucleic acid", "nucleic sequence", "nucleic acid sequence", "polynucleotide", "oligonucleotide", "polynucleotide sequence" and "nucleotide sequence", used interchangeably in the present description, mean a precise sequence of nucleotides, modified or not, defining a fragment or a region of a nucleic acid, containing unnatural nucleotides or not, and being either a double-strand DNA, a single-strand DNA or transcription products of said DNAs.

It should also be included here that the present invention does not relate to nucleotide sequences in their natural chromosomal environment, i.e., in a natural state. The sequences of the present invention have been isolated and/or purified, i.e., they were sampled directly or indirectly, for example by a copy, their environment having been at least partially modified. Isolated nucleic acids obtained by recombinant genetics, by means, for example, of host cells, or obtained by chemical synthesis should also be mentioned here.

"Nucleic sequences exhibiting a percentage identity of at least 80%, preferably 85%, 90%, 95% and 98%, after optimal alignment with a preferred sequence" means nucleic sequences exhibiting, with respect to the reference nucleic sequence, certain modifications such as, in particular, a deletion, a truncation, an extension, a chimeric fusion and/or a substitution, notably punctual. Preferably, these are sequences which code for the same amino acid sequences as the reference sequence, this being related to the degeneration of the genetic code, or complementarity sequences that are likely to hybridize specifically with the reference sequences, preferably under highly stringent conditions, notably those defined below.

Hybridization under highly stringent conditions means that conditions related to temperature and ionic strength are selected in such a way that they allow hybridization to be maintained between two complementarity DNA fragments. On a purely illustrative basis, the highly stringent conditions of the hybridization step for the purpose of defining the polynucleotide fragments described above are advantageously as follows.

DNA-DNA or DNA-RNA hybridization is carried out in two steps: (1) prehybridization at 42°C for three hours in phosphate buffer (20 mM, pH 7.5) containing 5X SSC (1X SSC corresponds to a solution of 0.15 M NaCl + 0.015 M sodium citrate), 50% formamide, 7% sodium dodecyl sulfate (SDS), 10X Denhardt's,

5% dextran sulfate and 1% salmon sperm DNA; (2) primary hybridization for 20 hours at a temperature depending on the length of the probe (i.e.: 42°C for a probe >100 nucleotides in length) followed by two 20-minute washings at 20°C in 2X SSC + 2% SDS, one 20–minute washing at 20°C in 0.1X SSC + 0.1% SDS. The last washing is carried out in 0.1X SSC + 0.1% SDS for 30 minutes at 60°C for a probe >100 nucleotides in length. The highly stringent hybridization conditions described above for a polynucleotide of defined size can be adapted by a person skilled in the art for longer or shorter oligonucleotides, according to the procedures described in Sambrook, *et al.* (Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory; 3rd edition, 2001).

The invention also relates to a vector comprising a nucleic acid as described in the invention.

The invention notably targets cloning and/or expression vectors that contain such a nucleotide sequence.

The vectors of the invention preferably contain elements which allow the expression and/or the secretion of nucleotide sequences in a given host cell. The vector thus must contain a promoter, translation initiation and termination signals, as well as suitable transcription regulation regions. It must be able to be maintained in a stable manner in the host cell and may optionally have specific signals which specify secretion of the translated protein. These various elements are selected and optimized by a person skilled in the art according to the host cell used. For this purpose, the nucleotide sequences can be inserted in self-replicating vectors within the chosen host or be integrative vectors of the chosen host.

Such vectors are prepared by methods typically used by a person skilled in the art and the resulting clones can be introduced into a suitable host by standard methods such as lipofection, electroporation, heat shock or chemical methods.

The vectors are, for example, vectors of plasmid or viral origin. They are used to transform host cells in order to clone or express the nucleotide sequences of the invention.

The invention also comprises host cells transformed by or comprising a vector as described in the present invention.

The host cell can be selected among prokaryotic or eukaryotic systems such as bacterial cells, for example, but also yeast cells or animal cells, notably mammal cells. Insect or plant cells can also be used.

The invention also relates to animals, other than man, that have a transformed cell according to the invention.

Another aspect of the invention relates to a method for the production of an antibody according to the invention, or one of its functional fragments, characterized in that said method comprises the following steps:

a) the culture in a medium of and the suitable culture conditions for a host cell according to the invention; and

b) the recovery of said antibody, or one of its functional fragments, thus produced from the culture medium or from said cultured cells.

The transformed cells according to the invention are of use in methods for the preparation of recombinant polypeptides according to the invention. Methods for the preparation of polypeptide according to the invention in recombinant form, characterized in that said methods use a vector and/or a cell transformed by a vector according to the invention, are also comprised in the present invention. Preferably, a cell transformed by a vector according to the invention is cultured under conditions that allow the expression of the aforesaid polypeptide and recovery of said recombinant peptide.

As already mentioned, the host cell can be selected among prokaryotic or eukaryotic systems. In particular, it is possible to identify the nucleotide sequences of the invention that facilitate secretion in such a prokaryotic or eukaryotic system. A vector according to the invention carrying such a sequence can thus be used advantageously for the production of recombinant proteins to be secreted. Indeed, the purification of these recombinant proteins of interest will be facilitated by the fact that they are present in the supernatant of the cellular culture rather than inside host cells.

The polypeptides of the invention can also be prepared by chemical synthesis. One such method of preparation is also an object of the invention. A person skilled in the art knows methods for chemical synthesis, such as solid-phase techniques (see notably Steward *et al.*, 1984, Solid phase peptides synthesis, Pierce Chem. Company, Rockford, 111, 2nd ed.) or partial solid-phase techniques, by condensation of fragments

or by conventional synthesis in solution. Polypeptides obtained by chemical synthesis and capable of containing corresponding unnatural amino acids are also comprised in the invention.

The antibodies, or the derived compounds or functional fragments of same, likely to be obtained by the method of the invention are also comprised in the present invention.

According to still another aspect, the present invention relates to an antibody as described above, characterized in that it is, in addition, capable of specifically binding to a human chemokine family receptor and/or capable of specifically inhibiting the signaling of such a receptor.

According to a novel embodiment, the invention relates to an antibody, or its derived compounds or functional fragments, consisting of an antibody that is bispecific in the sense that it comprises a second motif capable of interacting with any receptor implicated in the development of tumors, such as, for example, VEGFR, VEGF, EGFR, IGF–1R, HER2neu, HGF, cMET, FGF, tetraspanins, integrins, CXCR4 (other than the antibody of the present invention, i.e. targeting another epitope), CXCR7 or CXCR2.

The bispecific or bifunctional antibodies constitute a second generation of monoclonal antibodies in which two different variable regions are combined in the same molecule (Hollinger and Bohlen, 1999, Cancer and metastasis, rev. 18:411-419). Their utility was demonstrated in both diagnostic and therapeutic domains relative to their capacity to recruit new effector functions or to target several molecules on the surface of tumor cells; such antibodies can be obtained by chemical methods (Glennie MJ *et al.*, 1987, J. Immunol. 139, 2367–2375; Repp R. *et al.*, 1995, J. Hemat., 377-382) or somatic methods (Staerz U.D. and Bevan M.J., 1986, PNAS 83, 1453-1457; Suresh M.R. *et al.*, 1986, Method Enzymol., 121:210-228) but also, preferentially, by genetic engineering techniques that make it possible to force heterodimerization and thus facilitate the purification of the antibody sought (Merchand *et al.*, 1998, Nature Biotech., 16:677-681).

These bispecific antibodies can be constructed as whole IgG, bispecific Fab'2, Fab'PEG, diabodies or bispecific scFv, but also as a tetravalent bispecific antibody in which two binding sites are present for each antigen targeted (Park *et al.*, 2000, Mol. Immunol., 37(18):1123-30) or the fragments of same as described above.

In addition to an economic advantage given that the production and administration of a bispecific antibody are cheaper than the production of two specific antibodies, the use of such bispecific antibodies has the advantage of reducing the treatment's toxicity. Indeed, the use of a bispecific antibody makes it possible to decrease the overall quantity of circulating antibodies and, consequently, possible toxicity.

In a preferred embodiment of the invention, the bispecific antibody is a bivalent or tetravalent antibody.

Lastly, the present invention relates to the antibody described above, or its derived compounds or functional fragments, for use as a drug.

The invention also relates to a pharmaceutical composition comprising as an active ingredient a compound consisting of an antibody of the invention, or one of its derived compounds or functional fragments. Preferably, said antibody is supplemented by an excipient and/or a pharmaceutically acceptable carrier.

The invention also relates to a composition characterized in that it comprises, in addition, as a combination product for use in a simultaneous, separated or extended fashion, an anti-tumor antibody other that an antibody directed against CXCR4.

According to still another embodiment, the present invention also relates to a pharmaceutical composition as described above that comprises at least a second antitumor compound selected among the compounds capable of specifically inhibiting the tyrosine kinase activity of receptors such as IGF-IR, EGFR, HER2/neu, cMET, VEGFR or VEGF, or any other antitumor compound known to a person skilled in the art.

In a second preferred aspect of the invention, said second compound can be selected among the antibodies antiEGFR, antiIGF-IR, antiHER2/neu, anticMET, VEGFR, VEGF, etc., isolated, or their functional fragments and derived compounds, capable of inhibiting the proliferative and/or anti-apoptotic and/or angiogenic and/or inductive activity of metastatic dissemination promoted by said receptors.

Also suitable for mention are antiCD20 antibodies such as a rituximab, ibritumomab or tositumomab; antiCD33 antibodies such as gemtuzumab or lintuzumab; antiCD22 antibodies such as epratuzumab; antiCD52 antibodies such as alemtuzumab; antiEpCAM antibodies such as edrecolomab, Ch 17-1A or IGN-101; antiCTP21 or 16

antibodies such as Xactin; antiDNA-Ag antibodies such as ^{131}I-Cotara TNT-1; antiMUC1 antibodies such as pemtumomab or R1150; antiMUC18 antibodies such as ABX-MA1; antiGD3 antibodies such as mitumomab; antiECA antibodies such as CeaVac or labetuzumab; antiCA125 antibodies such as OvaRex; antiHLA-DR antibodies such as apolizumab; antiCTLA4 antibodies such as MDX-010; antiPSMA antibodies such as MDX-070, ^{111}In & ^{90}Y-J591, ^{177}Lu J591, J591-DM1; antiLewis Y antibodies such as IGN311; antiangiogenesis antibodies such as AS1405 and 90YmuBC1; antiTrail-R1 antibodies such as TRAIL R1mAb or TRAIL R2mAb.

Another embodiment complementary to the invention consists of a composition as described above comprised of, in addition, as a combination or conjugaison product for simultaneous, separated or extended use, a cytotoxic/cytostatic agent.

"Simultaneous use" means the administration of both compounds of the composition comprised in a single dosage form.

"Separated use" means administration, at the same time, of both compounds of the composition, comprised in distinct dosage forms.

"Extended use" means the successive administration of both compounds of the composition, each comprised in a distinct dosage form.

Generally, the composition according to the invention considerably increases cancer treatment effectiveness. In other words, the therapeutic effect of the antibody of the invention is enhanced in an unexpected way by the administration of a cytotoxic agent. Another major subsequent advantage produced by a composition of the invention relates to the possibility of using lower effective doses of the active ingredient, thus making it possible to avoid or reduce the risks of the appearance of side effects, in particular the effect of the cytotoxic agent. Moreover, this composition makes it possible to achieve the expected therapeutic effect more quickly.

"Therapeutic anticancer agent" or "cytotoxic agent" means a substance which, when it is administered to a patient, treats or prevents the development of cancer in the patient. Non-limiting examples of such agents include "alkylating" agents, antimetabolites, antitumor antibiotics, mitotic inhibitors, inhibitors of chromatin functioning, antiangiogenics, antiestrogens, antiandrogens and immunomodulators.

Such agents, for example, are cited in VIDAL, on the page devoted to compounds related to oncology and hematology under the heading "Cytotoxic"; the

cytotoxic compounds cited by reference to this document are cited herein as preferred cytotoxic agents.

"Alkylating agent" refers to any substance that can bind covalently with or can alkylate any molecule, preferentially a nucleic acid (e.g., DNA), within a cell. Examples of such alkylating agents include nitrogen mustards such as mechlorethamine, chlorambucil, melphalan, chlorhydrate, pipobroman, prednimustine, disodium phosphate or estramustine; oxazaphosphorines such as cyclophosphamide, altretamine, trofosfamide, sulfofosfamide or ifosfamide; aziridines or ethylene-imines such as thiotepa, triethyleneamine or altetramine; nitrosoureas such as carmustine, streptozocine, fotemustine or lomustine; alkyl sulfonates such as busulfan, treosulfan or improsulfan; triazenes such as dacarbazine; or platinum complexes such as cisplatine, oxaliplatine or carboplatine.

"Antimetabolite" refers to a substance that blocks growth and/or cellular metabolism by interfering with certain activities, generally DNA synthesis. Examples of antimetabolites include methotrexate, 5-fluorouracile, floxuridine, 5-fluorodeoxyuridine, capecitabine, cytarabine, fludarabine, cytosine arabinoside, 6-mercaptopurine (6-MP), 6-thioguanine (6-TG), chlorodesoxyadenosine, 5-azacytidine, gemcitabine, cladribine, deoxycoformycin and pentostatin.

"Antitumor antibiotic" refers to a compound that can prevent or inhibit the synthesis of DNA, RNA and/or proteins. Examples of such antitumor antibiotics include doxorubicin, daunorubicin, idarubicin valrubicin, mitoxantrone, dactinomycin, mithramycin, plicamycin, mitomycin C, bleomycin and procarbazine.

"Mitotic inhibiters" prevent the normal progression of the cell cycle and mitosis. In general, microtubule inhibiters or "taxoids" such as paclitaxel and docetaxel are capable of inhibiting mitosis. The vinca alkaloids, such as vinblastine, vincristine, vindesine and vinorelbine, are also capable of inhibiting mitosis.

"Chromatin inhibiters" or "topoisomerase inhibiters" are substances that inhibit the normal functioning of proteins that shape chromatin, such as topoisomerases I and II. Examples of such inhibiters include, for topoisomerase I, camptothecine and its derivatives, such as irinotecan or topotecan; for topoisomerase II, etoposide, etiposide phosphate and teniposide.

An "antiangiogenic" is any drug, compound, substance or agent that inhibits the growth of the blood vessels. Examples of antiangiogenics include, without being limiting, razoxin, marimastat, batimastat, prinomastat, tanomastat, ilomastat, CGS-27023A, halofuginone, COL-3, neovastat, BMS-275291, thalidomide, CDC 501, DMXAA, L–651582, squalamine, endostatine, SU5416, SU6668, interferon-alpha, EMD121974, interleukin-12, IM862, angiostatin and vitaxin.

"Antiestrogen" or "estrogen antagonist" refers to any substance that decreases, antagonizes or inhibits estrogen action. Examples of such agents are tamoxifene, toremifene, raloxifene, droloxifene, iodoxyfene, anastrozole, letrozole and exemestane.

"Antiandrogen" or "androgen antagonist" refers to any substance that reduces, antagonizes or inhibits androgen action. Examples of antiandrogens include flutamide, nilutamide, bicalutamide, sprironolactone, cyproterone acetate, finasteride and cimitidine.

Immunomodulators are substances that stimulate the immune system. Examples of immunomodulators include interferon, interleukins such as aldesleukin, OCT-43, denileukin diftitox or interleukine-2, tumor necrosis factors such as tasonermine, or other types of immunomodulators such as lentinan, sizofiran, roquinimex, pidotimod, pegademase, thymopentine, poly I:C or levamisole in combination with 5-fluorouracil.

For further details, a person skilled in the art can refer to the manual published by the French Association of Therapeutic Chemistry Teachers titled "Therapeutic chemistry, vol. 6, Antitumor drugs and perspectives in the treatment of cancer, TEC and DOC edition, 2003 [in French]".

In a particularly preferred embodiment, said composition of the invention as a combination product is characterized in that said cytotoxic agent is bound chemically to said antibody for use simultaneously.

In a particularly preferred embodiment, said composition is characterized in that said cytotoxic/cytostatic agent is selected among the spindle inhibitors or stabilizers, preferably vinorelbine and/or vinflunine and/or vincristine.

In order to facilitate binding between said cytotoxic agent and the antibody according to the invention, spacer molecules can be introduced between the two compounds to bind, such as the poly(alkylene)glycol polyethyleneglycol or the amino acids; or, in another embodiment, said cytotoxic agents' active derivatives, into which

have been introduced functions capable of reacting with said antibody, can be used. These binding techniques are well-known to a person skilled in the art and will not be discussed in more detail in the present description.

Other EGFR inhibiters include, without being limiting, monoclonal antibodies C225 and antiEGFR 22Mab (ImClone Systems Incorporated), ABX-EGF (Abgenix/Cell Genesys), EMD-7200 (Merck KgaA) or compounds ZD-1834, ZD-1838 and ZD-1839 (AstraZeneca), PKI-166 (Novartis), PKI-166/CGP-75166 (Novartis), PTK 787 (Novartis), CP 701 (Cephalon), flunomide (Pharmacia/Sugen), CI–1033 (Warner Lambert Parke Davis), CI-1033/PD 183, 805 (Warner Lambert Parke Davis), CL-387, 785 (Wyeth-Ayerst), BBR–1611 (Boehringer Mannheim GMBH/Roche), Naamidine A (Bristol-board Myers Squibb), RC-3940-II (Pharmacia), BIBX-1382 (Boehringer Ingelheim), OLX–103 (Merck & Co), VRCTC–310 (Ventech Research), EGF fusion toxin (Seragen Inc.), DAB-389 (Seragen/Lilgand), ZM–252808 (Imperial Cancer Research Fund), RG-50864 (INSERM), LFM-A12 (Parker Hughes Center Cancer), WHI–P97 (Parker Hughes Center Cancer), GW-282974 (Glaxo), KT–8391 (Kyowa Hakko) or the "EGFR vaccine" (York Medical/Centro of Immunologia Molecular).

Another aspect of the invention relates to a composition characterized in that at least one of said antibodies, or of the derived compounds or functional fragments of same, is combined or conjugated with a cellular toxin and/or a radioisotope.

Preferably, said toxin or said radioisotope is capable of preventing the growth or proliferation of the tumor cell, notably of completely inactivating said tumor cell.

Also preferably, said toxin is an enterobacteria toxin, notably *Pseudomonas* exotoxin A.

The radioisotopes preferentially combined with therapeutic antibodies are radioisotopes that emit gamma rays, preferentially iodine^{131}, yttrium^{90}, gold^{199}, palladium^{100}, copper^{67}, bismuth^{217} and antimony^{211}. Radioisotopes that emit alpha and beta rays can also be used in therapy.

"Toxin or radioisotope combined with at least one antibody of the invention, or a functional fragment of same" refers to any means that makes it possible to bind said toxin or said radioisotope to that at least one antibody, notably by covalent binding between the two compounds, with or without the introduction of the binding molecule.

Examples of agents that allow chemical (covalent), electrostatic, or non-covalent bonding of all or part of the conjugate's elements include, in particular, benzoquinone, carbodiimide and more particularly EDC (1-ethyl-3-[3-dimethyl-aminopropyl]-carbodiimide-hydrochloride), dimaleimide, dithiobis-nitrobenzoic (DTNB) acid, N-succinimidyl S-acetyl thio-acetate (SATA), bridging agents with one or more groups, with one or more phenylaside groups, reacting with ultraviolet (UV) rays, most preferentially N-[-4 (azidosalicylamino)butyl]-3'-(2'-pyridyldithio)-propionamide (APDP), N-succinimid-yl 3(2-pyridyldithio) propionate (SPDP) and 6-hydrazino-nicotinamide (HYNIC).

Another form of binding, notably for radioisotopes, can consist of the use of bifunctional ion chelating agents.

Examples of such chelators include the chelators derived from EDTA (ethylenediaminetetraacetic acid) or DTPA (diethylenetriaminepentaacetic acid) which were developed to bind metals, particularly radioactive metals, with immunoglobulins. Thus, DTPA and its derivatives can be substituted on the carbon chain by various groups in such a way as to increase the stability and the rigidity of the ligand-metal complex (Krejcarek *et al.*, 1977; Brechbiel *et al.*, 1991; Gansow, 1991; US patent 4,831,175).

For example, DTPA (diethylenetriaminepentaacetic acid) and its derivatives, which long have been widely used in drug and biology either in its free form or in a complex with a metal ion, exhibit the remarkable characteristic of forming stable chelates with metal ions which can be coupled with proteins of therapeutic or diagnostic interest, such as antibodies, for the development of radio-immuno conjugates for cancer therapy (Meases *et al.*, 1984; Gansow *et al.*, 1990).

Also preferably, said at least one antibody of the invention forming said conjugate is selected among its functional fragments, notably fragments that have lost their Fc component, such as scFv fragments.

The present invention also comprises the use of the composition for the preparation of a drug intended for the prevention or the treatment of cancer.

The present invention also relates to the use of an antibody, or a derived compound or functional fragment of same, preferably humanized, and/or of a composition according to the invention for the preparation of a drug for inhibiting the

growth of tumor cells. Generally, the present invention relates to the use of an antibody, or a derived compound or functional fragment of same, preferably humanized, and/or of a composition, for the preparation of a drug for cancer prevention or treatment.

Preferred cancers that can be prevented and/or treated include prostate cancer, osteosarcoma, lung cancer, breast cancer, endometrial cancer, colon cancer, multiple myeloma, ovarian cancer, pancreatic cancer or any other cancer.

The invention also concerns the use of an antibody, or a derived compound or functional fragment of same, and/or of a composition as above described for the preparation of a drug for modulating CXCR4 activity in a cell.

Another aspect of the present invention relates to the use of the antibody as described in a diagnostic method, preferably *in vitro*, of diseases related to CXCR4 expression level. Preferably, said CXCR4 protein related diseases in said diagnostic method will be cancers.

Thus, the antibodies of the invention, or the derived compounds or functional fragments of same, can be employed in a method for the detection and/or quantification of CXCR4 protein in a biological sample *in vitro*, notably for the diagnosis of diseases associated with an abnormal expression with this protein, such as cancers, wherein said method comprises the following steps:

a) placing the biological sample in contact with an antibody according to the invention, or a derived compound or functional fragment of same;

b) demonstrating the antigen-antibody complex possibly formed.

Thus, the present invention also comprises the kits or accessories for the implementation of a method as described, comprising the following elements:

a) a polyclonal or monoclonal antibody of the invention;

b) optionally, reagents for constituting the medium favorable to immunological reactions;

c) optionally, reagents that reveal the antigen-antibodies complexes produced by the immunological reaction.

Advantageously, the antibodies or functional fragments of same can be immobilized on a support, notably a protein chip. One such protein chip is an object of the invention.

Advantageously, the protein chips can be used in the kits or accessories required for detecting and/or quantifying CXCR4 protein in a biological sample.

It must be stated that the term "biological sample" relates herein to samples taken from a living organism (notably blood, tissue, organ or other samples taken from a mammal, notably man) or any sample likely to contain one such CXCR4 protein (such as a sample of cells, transformed if needed).

Said antibody, or a functional fragment of same, can be in the form of an immunoconjugate or of a labeled antibody in order to obtain a detectable and/or quantifiable signal.

The labeled antibodies of the invention, or the functional or fragments of same, include, for example, antibody conjugates (immunoconjugates), which can be combined, for example, with enzymes such as peroxidase, alkaline phosphatase, α-D-galactosidase, glucose oxidase, glucose amylase, carbonic anhydrase, acetyl-cholinesterase, lysozyme, malate dehydrogenase or glucose-6 phosphate dehydrogenase or by a molecule such as biotin, digoxigenin or 5-bromo-desoxyuridine. Fluorescent labels can be also combined with the antibodies of the invention or functional fragments of same, including notably fluorescein and its derivatives, fluorochrome, rhodamine and its derivatives, green fluorescent protein (GFP), dansyl, umbelliferone, etc. In such conjugates, the antibodies of the invention or functional fragments of same can be prepared by methods known to a person skilled in the art. They can be bound with enzymes or fluorescent labels directly; via a spacer group or a linkage group such as polyaldehyde, glutaraldehyde, ethylenediaminetetraacetic acid (EDTA) or diethylenetriaminepentaacetic acid (DPTA); or in the presence of binding agents such as those mentioned above for therapeutic conjugates. Conjugates carrying fluorescein labels can be prepared by reaction with an isothiocyanate.

Others conjugates can also include chemiluminescent labels such as luminol and dioxetane, bioluminescent labels such as luciferase and luciferin, or radioactive labels such as iodine^{123}, iodine^{125}, iodine^{126}, iodine^{133}, bromine^{77}, technetium^{99m}, indium^{111}, indium^{113m}, gallium^{67}, gallium^{68}, ruthenium^{95}, ruthenium^{97}, ruthenium^{103}, ruthenium^{105}, mercury^{107}, mercury^{203}, rhenium^{99m}, rhenium^{101}, rhenium^{105}, scandium^{47}, tellurium^{121m}, tellurium^{122m}, tellurium^{125m}, thulium^{165}, thulium^{167}, thulium^{168}, fluorine^{18}, yttrium^{199} and iodine^{131}. Existing methods known to a person skilled in the art for binding

radioisotopes with antibodies, either directly or via a chelating agent such as the EDTA or DTPA mentioned above, can be used for as diagnostic radioisotopes. Thus should be mentioned labeling with [I^{125}]Na by the chloramine-T technique [Hunter W.M. and Greenwood F.C. (1962) Nature 194:495]; labeling with technetium^{99m} as described by Crockford *et al.* (US patent 4,424,200) or bound via DTPA as described by Hnatowich (US patent 4,479,930).

The invention also relates to the use of an antibody according to the invention for the preparation of a drug for the specific targeting of a compound that is biologically active toward cells expressing or overexpressing CXCR4.

In the sense of the present description, a "biologically active compound" is any compound capable of modulating, notably inhibiting, cellular activity, notably growth, proliferation, transcription and gene translation.

The invention also relates to an *in vivo* diagnostic reagent composed of an antibody according to the invention, or a functional fragment of same, preferably labeled, notably radiolabeled, and its use in medical imaging, notably for the detection of cancer related to the cellular expression or overexpression of CXCR4.

The invention also relates to a composition as a combination product or to an anti-CXCR4/toxin conjugate or radioisotope, according to the invention, used as drug.

Preferably, said composition as a combination product or said conjugate will be supplemented by an excipient and/or a pharmaceutical vehicle.

In the present description, "pharmaceutical vehicle" means a compound, or a combination of compounds, entering a pharmaceutical composition that does not cause secondary reactions and that, for example, facilitates administration of the active compounds, increases its lifespan and/or effectiveness in the organism, increases its solubility in solution or improves its storage. Such pharmaceutical carriers are well-known and will be adapted by a person skilled in the art according to the nature and the administration route of the active compounds selected.

Preferably, such compounds will be administered by systemic route, notably by intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous or oral route. More preferably, the composition composed of the antibody according to the invention will be administered in several doses spaced equally over time.

Their administration routes, dosing schedules and optimal galenic forms can be determined according to the criteria generally taken into account when establishing a treatment suited to a patient such as, for example, the patient's age or body weight, the seriousness of his general state, his tolerance for the treatment and the side effects experienced.

Thus, the invention relates to the use of an antibody, or one of its functional fragments, for the preparation of a drug for the specific targeting of a compound that is biologically active toward cells expressing or overexpressing CXCR4.

Other characteristics and advantages of the invention appear further in the description with the examples and figures whose legends are presented below.

FIGURE LEGENDS

Figures 1A and 1B show CXCR4 and CXCR2 expression in cancer cells by qPCR analysis, respectively.

Figure 2 shows CXCR4 and CXCR2 protein expression in cancer cells by FACS analysis.

Figures 3A and 3B show the competition of specific [^{125}I]SDF1 binding by unlabeled SDF-1 (Figure 3A) and 414H5 and 515H7 Mabs (Figure 3B) on cellular membranes of CHO-K1 cells stably expressing wild-type human CXCR4 (T: total binding ; NS: non-specific binding).

Figures 4A and 4B show the modulation of G protein activation by 414H5 Mab (Figure 4A) and 515H7 Mab (Figure 4B) by monitoring [^{35}S]GTPγS binding responses at wild-type CXCR4 receptor stably expressed in NIH-3T3 cells.

Figure 5 shows the modulation of G protein activation by anti-CXCR4 Mabs 414H5 and 515H7 by monitoring [^{35}S]GTPγS binding responses at HeLa human tumor cells stimulated with SDF-1 (10 and 100 nM).

Figures 6A-6F show the modulation of CXCR4 receptor association with different interaction partners by SDF-1 and by 414H5 and 515H7 Mabs via a bioluminescence resonance energy transfer (BRET) approach in HEK293 cells. (Figures 6A and 6B: CXCR4:CXCR4 homo-dimerization; Figures 6C and 6D: CXCR2:CXCR4

hetero-dimerization and Figures 6E and 6F: CXCR4-mediated recruitment of β-arrestin).

Figures 7A and 7B show the inhibition of forskolin-stimulated cAMP production by SDF-1 and 414H5 and 515H7 Mabs in NIH3T3 cells stably expressing CXCR4 receptor.

Figure 8 shows the modulation of G protein activation by anti-CXCR4 Mabs 414H5 and 515H7 by monitoring [^{35}S]GTPγS binding responses at constitutively active mutant Asn^{119}Ser CXCR4 receptor stably expressed in CHO-K1 cells.

Figure 9 illustrates the inhibition of SDF-1 induced Hela cell proliferation by the Mab 414H5 *in vitro*.

Figures 10A and 10B show the inhibition of SDF-1-induced U937 cells migration by CXCR4 Mab 414H5 (Figure 10A) and Mab 515H7 (Figure 10B) *in vitro*.

Figure 11 shows inhibition of MDA-MB-231 xenograft tumor growth by anti-CXCR4 Mab 414H5 (A) and Mab 515H7 (B) in Nod/Scid mice.

Figure 12 shows the anti-CXCR4 Mab 414H5 activity in U937 Nod/Scid mice survival model.

Figures 13A-13C show the SDF-1-induced calcium release inhibition by anti-CXCR4 Mab 515H7 in CHO-CXCR4 cells (Figure 13A) and MDA-MB-231 (Figure 13B), U937 (Figure 13C) cancer cells.

Figure 14 shows the inhibition of T-cell KARPAS 299 xenograft tumor in Nod/Scid mice by 414H5.

Figure 15 shows the activity of murine anti-CXCR4 Mab m515H7 in U937 Nod/Scid mice survival model.

Figure 16 shows the activity of murine anti-CXCR4 Mab m515H7 in inhibition of T-cell KARPAS 299 xenograft tumor growth in Nod/Scid mice

Figure 17 shows the competition of specific [^{125}I]SDF1 binding by murine m414H5 and m515H7 Mabs and chimeric Mabs c414H5 and c515H7 on cellular membranes of CHO-K1 cells stably expressing wild-type human CXCR4 (T: total binding ; NS: non-specific binding).

Figure 18 shows the modulation of G protein activation by murine m414H5 and m515H7 Mabs and by chimeric Mabs c414H5 and c515H7 by monitoring [^{35}S]GTPγS

binding responses at wild-type CXCR4 receptor stably expressed in NIH-3T3 cells stimulated with SDF-1 (10 nM).

Figure 19 shows the modulation of G protein activation by anti-CXCR4 murine m414H5 and m515H7 Mabs and chimeric Mabs c414H5 and c515H7 by monitoring [^{35}S]GTPγS binding responses at HeLa human tumor cells stimulated with SDF-1 (10 nM).

Figures 20A-20C show the modulation of CXCR4 receptor association with different interaction partners by SDF-1 and by m414H5, c414H5, m515H7 and c515H7 Mabs via a bioluminescence resonance energy transfer (BRET) approach in HEK293 cells. (Figure 20A: CXCR4:CXCR4 homo-dimerization; Figure 20B: CXCR2:CXCR4 hetero-dimerization and Figures 20C: CXCR4-mediated recruitment of β-arrestin).

Figures 21A and 21B show the inhibition of SDF-1-induced calcium release in CHO-CXCR4 cells (figure 21A) and in U937 cells (figure 21B)

Figures 22A and 22B show the inhibition of SDF-1-induced U937 cells migration by CXCR4 Mabs m414H5 and c414H5 (Figure 22A) and Mabs m515H7 and c515H7 (Figure 22B) *in vitro*.

Figure 23 shows the anti-CXCR4 chimeric Mabs c414H5 and c515H7 activity in U937 Nod/Scid mice survival model.

EXAMPLES

Example 1: Expression of CXCR4 and CXCR2 in cancer cells

- Q-PCR analysis:

In order to quantify the relative expression of CXCR4 and CXCR2 in different cancer cell lines, a real time RT-PCR was used.

RNA samples were extracted from different cell lines using RNeasy Mini or Midi Protocols (Qiagen Corporation, France). The RNA samples were then controlled using the Experion automated electrophoresis system (BIO-RAD Corporation, France) and showed a good quality/integrity. One µg of each RNA sample was converted into cDNA template using the iScript cDNA Synthesis kit (BIO-RAD Corporation, France). cDNA levels were quantified using qPCR with either a TaqMan probe for CXCR2 or SYBERGreen for CXCR4. Comparing samples requires normalization, so was introduced the internal reference RPL0. TaqMan probes (used for CXCR2) carried a 5'

FAM reporter label and a 3' TAMRA quencher group. The PCR enzyme was activated by heating for 2 min at 50°C and 10 min at 95°C. A two step procedure was used, 15 sec at 95°C and 1 min at 62°C for 40 or 45 cycles in a PCR mix containing 5 µl of cDNA template (dilution 1/20), 1 x qPCR Mastermix (TaqMan Universal PCR Master Mix, Applied Biosystems corporation, Branchburg New Jersey, USA), 50 to 900 nM of each primers and 50 to 100 nM probe in a total volume of 50 µl. All reactions were performed using iCycler instrument (BIO-RAD Corporation). Q-PCR allowed to determine Cycle threshold (Ct). The more the Ct value is small, the more the gene tested is expressed. Primers and probe for Human Ribosomal protein, large, P0 were:

forward primer, 5'-GAAACTCTGCATTCTCGCTTCCTG-3' (SEQ ID No.32);

reverse primer, 5'-AGGACTCGTTTGTACCCGTTGA-3'(SEQ ID No.33);

probe, 5 -(FAM)- TGCAGATTGGCTACCCAACTGTTGCA-(TAMRA)-3' (SEQ ID No. 34).

Primers for Human CXCR4 (chemokine receptor 4) were:

forward primer, 5'-CTCCTTCATCCTCCTGGAAATC-3' (SEQ ID No. 35);

reverse primer, 5'-CCAAGGAAAGCATAGAGGATGG-3' (SEQ ID No. 36).

Primers and probe for Human CXCR2 (chemokine receptor 2) were:

forward primer, 5'-GTGGTCATTATCTATGCCCTGG-3' (SEQ ID No. 37);

reverse primer, 5'-CGACCCTGCTGTATAAGATGAC-3' (SEQ ID No.38);

probe, 5 -(FAM)- TATTCCTGCTGAGCCTGCTGGGAAA-(TAMRA)-3' (SEQ ID No. 39).

In our comparative study, the expression of two genes [the gene tested (CXCR4 or CXCR2) and RPL0]) were quantified in two different samples: the cell line tested and a reference cell line. The reference cell line corresponded to the cell line containing the lowest expression of the gene quantified. Comparative gene expression calculation was made using the following formula:

Relative gene expression = $(1+E_{gene})^{-\Delta Ct(1)} / (1+E_{RPL0})^{-\Delta Ct(2)}$

E_{gene} = PCR efficiency using primers/probe of the gene quantified

E_{RPL0} = PCR efficiency using the RPL0 primers/probe

Ct = threshold cycle

$\Delta Ct(1) = Ct_{gene}$ (cell line tested) - Ct_{gene} (reference cell line)

$\Delta Ct(2) = Ct_{RPL0}$ (cell line tested) - Ct_{RPLO} (reference cell line)

For each PCR series, a relative gene quantity value was calculated, and cancer cell lines were classified into groups considering their levels of expression from the highest to negative. All data are presented in Figures 1A and 1B. All the cancer cell lines tested expressed CXCR4 (Figure 1A) and CXCR2 excepted DU145 and U-87MG for CXCR2 (Figure 1B).

- FACS analysis:

MDA-MB-231, PC3 and U937 cancer cell lines were permebilized and then incubated with either 10 µg/mL of anti-CXCR4 monoclonal antibodies [44717 (R&D Systems) versus its isotype control IgG2b (SIGMA] or 10 µg/mL of anti-CXCR2 monoclonal antibodies (anti h-CXCR2, clone 48311, R&D Systems, Mab 331 versus its isotype control IgG2a). The cells were then washed with 1%BSA/PBS/0.01% NaN3. Next, Alexa-labeled secondary antibodies were added to the cells and were allowed to incubate at 4°C for 20 min. Cells were then washed again two times. Following the second wash, FACS analysis was performed. Results of these binding studies are provided in Figure 2. Thus, tumor cells such as MDA-MB-231, PC3 and U937 expressed both CXCR4 and CXCR2 proteins.

Example 2: Generation of monoclonal antibodies (Mabs) against human CXCR4

To generate monoclonal antibodies to CXCR4, Balb/c mice were immunized with recombinant NIH3T3-CXCR4 cells and/or peptides corresponding to CXCR4 extracellular N-term and loops. The mice 6-16 weeks of age upon the first immunization, were immunized once with the antigen in complete Freund's adjuvant subcutaneously (s.c.) followed by 2 to 6 immunizations with antigen in incomplete Freund's adjuvant s.c. The immune response was monitored by retroorbital bleeds. The serum was screened by ELISA (as described bellow) and mice with the higher titers of anti-CXCR4 antibodies were used for fusions. Mice were boost intravenously with antigen two days before sacrifice and removal of the spleen.

- ELISA

To select the mice producing anti-CXCR4 antibodies, sera from immunized mice was tested by ELISA. Briefly, microtiter plates were coated with purified [1-41] N-terminal peptide conjugated to BSA at 5µg equivalent peptide/mL, 100µL/well

incubated at 4°C overnight, then blocked with 250µL/well of 0.5% gelatine in PBS. Dilutions of plasma from CXCR4-immunized mice were added to each well and incubated 2 hours at 37°C. The plates were washed with PBS and then incubated with a goat anti-mouse IgG antibody conjugated to HRP (Jackson Laboratories) for 1 hour at 37°C. After washing, plates were developed with TMB substrate, the reaction was stopped 5 min later by addition of 100 µL/well 1M H_2SO_4. Mice that developed the highest titers of anti-CXCR4 antibodies were used for antibody generation.

- Generation of hybridomas producing Mabs to CXCR4

The mouse splenocytes, isolated from a Balb/c mice that developed the highest titers of anti-CXCR4 antibodies were fused with PEG to a mouse myeloma cell line Sp2/O. Cells were plated at approximately 1x 10^5 /well in microtiter plates followed by two weeks incubation in selective medium containing ultra culture medium + 2 mM L-glutamine + 1 mM sodium pyruvate + 1x HAT. Wells were then screened by ELISA for anti-CXCR4 monoclonal IgG antibodies. The antibody secreting hybridomas were then subcloned at least twice by limiting dilution, cultured *in vitro* to generate antibody for further analysis.

Example 3: Characterization by FACS analysis of anti-CXCR4 Mabs 414H5 and 515H7 binding specificity and cancer cell lines recognition

In this experiment, specific binding to human CXCR4 of anti-CXCR4 Mabs 414H5 and 515H7 was examined by FACS analysis.

NIH3T3, NIH3T3-hCXCR4 transfected cells, MDA-MB-231, Hela and U937 cancer cell lines were incubated with 10 µg/mL of monoclonal antibody 414H5 and 515H7. The cells were then washed with 1%BSA/PBS/0.01% NaN3. Next, Alexa-labeled secondary antibodies were added to the cells and were allowed to incubate at 4°C for 20 min. The cells were then washed again two times. Following the second wash, FACS analysis was performed. Results of these binding studies are provided in the following Table 4 which shows [Mean Fluorescence Intensity (MFI) obtained by FACS] that anti-CXCR4 Mabs 414H5 and 515H7 bound specifically to human CXCR4-NIH3T3 transfected cell line whereas there was no recognition on the parent NIH3T3 cells. These Mab were also able to recognize human cancer cell lines, for examples

MDA-MB-231 breast cancer cells, U937 promyelocytic cancer cells and Hela cervix cancer cells.

Anti-CXCR4 Mabs 414H5 and 515H7 recognized NIH3T3-hCXCR4 transfectant while there was no recognition of the parent NIH3T3 wild type cells. Mabs 414H5 and 515H7 were also able to recognize cancer cell lines.

Table 4

Clone (10 µg/ml)	MFI on cell lines				
	NIH3T3	NIH3T3-CXCR4	MDA-MB-231	Hela	U937
414H5	21	2162	32	467	95
515H7	16	2752	239	1851	645

Example 4: Competition binding of anti-CXCR4 Mabs 414H5 and 515H7 for [^{125}I]SDF-1 at CHO-K1 membranes stably expressing human CXCR4 receptor

This assay allows to evaluate the ability of 414H5 and 515H7 Mabs to compete for binding of radio labeled [^{125}I]SDF-1 to human CXCR4 receptor, at either orthosteric or allosteric binding sites.

CHO-K1 cells, stably and constitutively expressing human CXCR4 receptor were obtained upon transfection of naïve CHO-K1 cells (ATCC CCL-61) with a mammalian expression vector carrying the whole coding sequence of human CXCR4 receptor (RefSeq NM_003467). Cells were propagated in complete culture medium [DMEM-Ham's F12 supplemented with 5 % fetal calf serum (FCS) and 500 µg/ml of geneticin]. Radioligand binding experiments were conducted on cell membranes obtained upon mechanical scrapping of CHO/CXCR4 cells in lysis buffer [Hepes 20mM, pH 7.4, NaCl 150mM] followed by centrifugation (10000g, 15 min). [^{125}I]SDF-1 binding (specific activity: 1500 Ci/mmol) was performed using the SPA technology (scintillation proximity assay – GE Healthcare). Briefly, cell membranes (30 µg/well) were incubated in binding buffer [Hepes 20mM, pH 7.4, $CaCl_2$ 1mM, $MgCl_2$ 5mM, NaCl 150mM, BSA 1%] together with compound to evaluate (SDF-1 or mAb), radioligand (1 nM) and finally SPA-WGA-PVT beads (7.3 mg/well). Binding equilibrium was reach after 1H at 25 °C. Upon centrifugation [1000 g for 10 min.]

radioactive counts were measured in a scintillation counter (TopCount, Perkin Elmer). Non-specific binding was estimated in the presence of 10 μM of unlabelled SDF-1.

Unlabelled SDF-1 dose-dependently inhibited [^{125}I]SDF-1 binding with a pKi value (IC_{50} = ligand concentration yielding 50 % inhibition of specific [^{125}I]SDF-1 binding) of 7.75 ± 0.27 nM (n=4) (Figure 3A). Under the same experimental conditions, our anti-CXCR4 Mabs (100 nM) efficiently competed for [^{125}I]SDF-1 binding with the following rank order of competition efficacy (% inhibition of [^{125}I]SDF-1): 515H7 (64±3%) 414H5 (43±4 %) (Figure 3B).

Example 5: Modulation of [^{35}S]GTPγS binding at cellular membranes expressing wild type CXCR4 receptor by anti-CXCR4 Mabs 414H5 and 515H7

This functional assay allows to monitor G protein activation via wild type human CXCR4 receptor and its modulation by CXCR4 ligands and 414H5 and 515H7 mAbs.

NIH-3T3 cells stably and constitutively expressing wild-type CXCR4 receptor were obtained as described in the example above for CHO-K1 cells. HeLa (human cervix carcinoma) cells were propagated in complete culture medium [EMEM supplemented with 10% FCS, 1% L-glutamine, 2 μ sodium bicarbonate]. [^{35}S]GTPγS binding was performed on cellular membranes obtained upon mechanical scrapping in lysis buffer [Hepes 20mM, pH 7.4, NaCl 150mM] and further centrifugation (10000 g, 15 min). Incorporation and detection of [^{35}S]GTPγS (specific activity: 1000 Ci/mmol) was performed using the SPA technology (scintillation proximity assay - GE Healthcare). Briefly, cell membranes (10 μg/well) were incubated in binding buffer [Hepes 20mM, GDP 3μM, $MgCl_2$ 10mM, NaCl 100mM, EDTA 1mM, pH=7.4] together with compound to evaluate (SDF-1 or Mab of interest), [^{35}S]GTPγS (0.2-0.4 nM) and finally SPA-WGA-PVT beads (7.3 mg/well). Binding reaction was performed during 1H at 25°C. Upon centrifugation [1000 g for 10 min.] radioactive counts were measured in a scintillation counter (TopCount, Perkin Elmer). Antagonist potency was calculated by applying the Cheng Prussof equation:

K_B= [conc antago]/{(EC_{50}'/EC_{50})-1} where EC_{50} and EC_{50}' are respectively the potency of SDF-1 in the absence and presence of mAb.

SDF-1 induced a dose-dependent increase of [^{35}S]GTPγS binding, as the result of G protein activation by CXCR4 receptor. Maximal stimulation of [^{35}S]GTPγS binding represents respectively 167 % and 320 % over basal [^{35}S]GTPγS binding for HeLa and NIH3T3/CXCR4 cell membranes. The potency of SDF-1 was similar for both cell lines and corresponded to 41.3 ± 9.7 nM (Figures 4A-4B). Under these experimental conditions, the antagonist potency of 414H5 and 515H7 Mabs, as determined in NIH3T3/CXCR4 cells was 51 nM and 15 nM, respectively. Similar antagonist efficacy was observed for HeLa cells (Figure 5).

Example 6: Association of CXCR4 with different interaction partners: homo and heterodimerization, recruitment of β-arrestin via a bioluminescence resonance energy transfer (BRET) approach and effect of 414H5 and 515H7 Mabs on these dimers

This functional assay allows to evaluate the conformational changes induced upon SDF-1 and/or 414H5 and 515H7 Mabs binding to CXCR4 receptor at the level of CXCR4 homo-dimer and CXCR2/CXCR4 hetero-dimer formation as well as the recruitment of the β-arrestin-2 signaling protein.

Expression vectors for each of the investigated interaction partners were constructed as fusion proteins with the corresponding dye (*Renilla reniformis* luciferase, Rluc and Yellow fluorescent protein, YFP) by applying conventional molecular biology techniques. Two days prior performing BRET experiments, HEK293 cells were transiently transfected with expression vectors coding for the corresponding BRET partners: [CXCR4/Rluc + CXCR4/YFP] to study CXCR4 homo dimerization, [CXCR4/Rluc + CXCR2:YFP] to study CXCR4 and CXCR2 hetero-dimerization and [CXCR4/Rluc + β-arr2:YFP] to study CXCR4-mediated recruitment of β-arrestin-2. The day after, cells were distributed in poly-lysine pre-coated white 96 MW plates in complete culture medium [DMEM supplemented with 10 % FBS]. Cells were first cultivated at 37°C with CO_2 5 % in order to allow cell attachment to the plate. Cells were then starved with 200 µl DMEM/well overnight. Immediately prior to the BRET experiment, DMEM was removed and cells were quickly washed with PBS. Cells were then incubated in PBS in the presence or absence of antibody, 10 min at 37°C prior to the addition of coelenterazine H 5 µM with or without SDF-1 300 nM in a final volume

of 50 µl. After incubation for further 10 minutes at 37°C, light-emission acquisition at 485 nm and 530 nm was initiated using the Mithras LB940 multilabel reader (Berthold) (1s/wavelength/well repeated 15 times at room temperature).

Calculation of BRET ratio was performed as previously described (Angers et al., 2000): [(emission$_{530\ nm}$) - (emission$_{485\ nm}$) X Cf] / (emission$_{485\ nm}$), where Cf = (emission$_{530\ nm}$) / (emission$_{485\ nm}$) for cells expressing the Rluc fusion protein alone under the same experimental conditions. Simplifying this equation shows that BRET ratio corresponds to the ratio 530/485 nm obtained when the two BRET partners are present, corrected by the ratio 530/485 nm obtained under the same experimental conditions, when only the partner fused to Rluc is present in the assay. For sake of readability, results are expressed in milliBRET units (mBU); mBU corresponds to the BRET ratio multiplied by 1000.

SDF1 (300 nM) increased by about 20 % the BRET signal resulting from the spatial proximity of the adaptor and acceptor proteins fused to CXCR4 receptor, it is likely to indicate CXCR4/CXCR4 homo-dimers formation or conformational changes of pre-existing dimers (Figures 6A and 6B). Interestingly, SDF1 (300 nM) decreased by about 24 % the BRET signal resulting from the spatial proximity of the adaptor and acceptor proteins fused to CXCR2 and CXCR4, likely indicating as well CXCR2/CXCR4 hetero-dimers formation or conformational changes of pre-existing dimers (Figures 6C and 6D). In this latter case, SDF-1-activated conformation of CXCR4/CXCR2 seems less favorable for BRET energy transfer. In both cases, 414H5 and 515H7 Mabs were able to modulate SDF-1-induced conformational changes for CXCR4 homo-dimers (63 % inhibition of SDF-1-induced BRET increase for 414H5 and 69 % inhibition of SDF-1-induced BRET increase for 515H7, Figures 6A and 6B, respectively) as well as for CXCR2/CXCR4 hetero-dimer formation (50 % inhibition of SDF-1-induced BRET decrease for 414H5 and 90 % inhibition of SDF-1-induced BRET decrease for 515H7, Figures 6C and 6D, respectively). 414H5 and 515H7 Mabs were also able to modulate by themselves CXCR4/CXCR4 and CXCR2/CXCR4 spatial proximity respectively, indicating an influence of 414H5 and 515H7 Mabs on both CXCR4/CXCR4 homo and CXCR2/CXCR4 hetero-dimer conformation. (Figures 6A, 6B, 6C and 6D).

CXCR4 activation by SDF-1 (300 nM) yielded a strong recruitment of the intracellular signaling molecule β-arrestin, as shown by the 233 % enhancement in BRET signal (Figures 6E and 6F). This recruitment was partially inhibited by 414H5 and 515H7 Mabs (about 20 % inhibition for 414H5 and 95 % for 515H7, Figures 6E and 6F, respectively) showing the effect of Mabs 414H5 and 515H7 on signaling.

Example 7: CXCR4-mediated inhibition of cAMP production

This functional assay was designed to monitor CXCR4 receptor signaling at the level of adenylate cyclases via inhibitory Gi/o proteins.

The cAMP LANCE procedure (Perkin Elmer) was applied as detailed by the supplier. Briefly, NIH3T3 cells stably and constitutively expressing wild type CXCR4 receptor were obtained and propagated as described above. Cells were collected using the trypsin-free agent Versene and resuspended at a concentration of 10^6 cells/ml in a solution containing the AlexaFluor-bound anti cAMP Mab ($1/100^{th}$ dilution) and compound (forskolin, SDF-1 and/or 414H5 and 515H7 Mabs). Upon incubation for 30 min. at room temperature, the detection mix containing the Europium-Streptavidin ($1/125^{th}$ dilution) and Biotin-cAMP ($1/125^{th}$ dilution) complexes was added. Upon incubation for 1 hour at room temperature, the resulting FRET signal was measured in a Mithras LB940 (Berthold) multilabel reader. Data are expressed either as arbitrary fluorescent values or as a relative stimulation versus SDF-1 response upon substraction of the FK effect.

Forskolin (FK) dose-dependently stimulated cAMP production with a potency of about 0.3 μM in NIH3T3/CXCR4 cells (Figure 7A). In the co-presence of SDF-1, intracellular cAMP levels decreased as a result of inhibitory Gi/o protein activation by CXCR4 receptor. The potency of SDF-1 was 5.0 ± 3.1 nM (Figure 7A). 414H5 and 515H7 Mabs efficiently inhibited the forskolin-stimulated effect of SDF-1 (100 nM) by more than 60 % for 414H5 and by more than 80 % for 515H7 (Figure 7B).

Example 8: Modulation of [^{35}S]GTPγS binding at cellular membranes expressing constitutively active mutant Asn^{119}Ser CXCR4 receptor by Mabs 414H5 and 515H7

This functional assay allows to monitor G protein activation via a constitutively active mutant (CAM) $Asn^{119}Ser$ CXCR4 receptor (see Zhang et al., 2002). This sensitive assay allows to discriminate CXCR4 ligands based on their intrinsic activity (partial agonist, silent antagonist or inverse agonist). As previously described by Zhang and colleagues, CXCR4 ligands such as AMD3100 or T140 behaved respectively as partial agonist and inverse agonist at CAM CXCR4 receptor. Identification of silent antagonist may be difficult since this class of molecule must display similar affinities for both active and inactive states of CXCR4 (Wurch et al., 1999).

Introduction of an Asn119Ser mutation in the coding sequence of CXCR4 receptor was performed by applying conventional molecular biology techniques (QuickChange site directed mutagenesis kit, Stratagene US). CHO-K1 cells stably and constitutively expressing CAM CXCR4 receptor were obtained as described in the example above. [^{35}S]GTPγS binding was performed on cellular membranes obtained upon mechanical scrapping in lysis buffer [Hepes 20mM, pH 7.4, NaCl 150mM] and further centrifugation (10000 g, 15 min). Incorporation of [^{35}S]GTPγS (specific activity: 1000 Ci/mmol) was performed using the SPA technology (scintillation proximity assay – GE Healthcare). Briefly, cell membranes (10 µg/well) were incubated in binding buffer [Hepes 20mM, GDP 3µM, $MgCl_2$ 10mM, NaCl 100mM, EDTA 1mM, pH=7.4] together with compound to evaluate (SDF-1 or mAb), [^{35}S]GTPγS (0.2-0.4 nM) and finally SPA-WGA-PVT beads (7.3 mg/well). Binding reaction was performed during 1H at 25 °C. Upon centrifugation [1000 g for 10 min.] radioactive counts were measured in a scintillation counter (TopCount, Perkin Elmer).

SDF-1 (100 nM) stimulated [^{35}S]GTPγS binding by 130 %. The inverse agonist T140 inhibited both basal (- 17 %) and SDF-1-stimulated (- 159 %) [^{35}S]GTPγS binding. In contrast, 414H5 and 515H7 Mabs behaved as silent antagonists at CAM CXCR4, without altering basal [^{35}S]GTPγS binding (Figure 8) but inhibiting SDF-1 induced [^{35}S]GTPγS binding (Figure 8).

Example 9: Inhibition of SDF-1-induced Hela cell proliferation by CXCR4 Mab 414H5 *in vitro*

HeLa cells from ATCC were routinely cultured in EMEM medium (Lonza Corporation. Verviers. Belgium), 10% FCS (SIGMA Corporation. St Louis. USA), 1%

L-Glutamine (Invitrogen Corporation. Scotland. UK), 2% Sodium bicarbonate 7.5% solution (Invitrogen Corporation. Scotland. UK). Cells were split 3 days before proliferation assays so that they were confluent.

- SDF-1-induced Hela cell proliferation

HeLa cells were plated in 96-well tissue culture plates at a density of 1 x 10^4 cells/well in 200 µl of serum free medium (EMEM medium plus 1% L-Glutamine. 2% sodium bicarbonate 7.5% solution). Twenty four hours after plating, appropriate dilutions of SDF-1 were added to HeLa cells. After a total of 76 hours of culture, cells were pulsed with 0.25 µCi of [^{3}H]thymidine (Amersham Biosciences AB. Uppsala. Sweden) for 16 hours. The magnitude of [^{3}H]thymidine incorporated in DNA was quantified by liquid scintillation counting.

Results were expressed as proliferation Index = [mean cpm of cells + SDF-1 / mean cpm of cells - SDF-1].

HeLa cells were incubated with SDF-1 (0 to 1000ng/ml). SDF-1 stimulated *in vitro* HeLa cells proliferation 1.5 to 2 folds. The concentration of SDF-1 to obtain the highest and reproducible proliferation index was 200 ng/ml (25 nM).

- Inhibition of SDF-1-induced Hela cell proliferation in vitro by CXCR4 414H5 Mab

HeLa cells were plated in 96-well tissue culture plates at a density of 1 x 10^4 cells/well in 200 µl of serum free medium (EMEM medium plus 1% L-Glutamine. 2% sodium bicarbonate 7.5% solution). Twenty four hours after plating, appropriate dilutions of anti-CXCR4 Mab 414H5, diluting media were added in triplicate to HeLa cells either in presence or in absence of SDF-1 at a final concentration of 200 ng/ml (25 nM). After a total of 76 hours of culture, cells were pulsed with 0.25 µCi of [^{3}H]thymidine (Amersham Biosciences AB. Uppsala. Sweden) for 16 hours. The magnitude of [^{3}H]thymidine incorporated in DNA was quantified by liquid scintillation counting.

Results were expressed as affected fraction (Fa) calculated using the formula:

Fa = [1- [mean cpm of cells incubated with Mab + SDF-1 / mean cpm of cells incubated with diluting media + SDF-1)] x 100.

The *in vitro* effect of anti-CXCR4 Mab 414H5 on SDF-1-induced HeLa cell proliferation was characterized. HeLa cells were incubated with either 414H5 Mab or

control with or without SDF-1 (200 ng/ml). SDF-1 stimulated the *in vitro* growth of HeLa cells (1.5 to 2 folds). Dose-response curve for 414H5 Mab was obtained by treating cells with serial two fold dilutions of Mab ranging from 0 to 1500 nM 24 h after cell plating. As cell proliferation was evaluated 76 hours after plating, each tested condition corresponds to a 48 h exposure time to Mab or control. Results were expressed as affected fraction using Fa formula described above. The results (represented in figure 9) showed that CXCR4 Mab 414H5 inhibited SDF-1-induced Hela cell proliferation *in vitro*.

Example 10: Effect of anti-CXCR4 Mabs 414H5 and 515H7 on SDF-1-induced U937 cells migration

To evaluate the inhibiting effect of the anti-CXCR4 monoclonal antibodies 414H5 and 515H7 on the migration process, 100 000 U-937 cells in RPMI 1640 medium supplemented with 2% FCS, were plated in the upper chamber of migration chambers (24 wells plates with 8-µm pore size) either in presence or in absence of SDF-1 in the lower part of the wells and with or without Mabs 414H5 and 515H7 in the upper chamber. In this test murine IgG2a and IgG2B were introduced as an isotype controls. Two hours after plating, migrating cells were counted. The results presented in figures 10A for 414H5 and 10B for 515H7 demonstrated that, as expected SDF-1 was able to induce a significant increase of U-937 cells migration. No effect was observed when cells were incubated with the IgG2 isotype control. In contrast, for cells incubated with the 414H5 and 515H7 Mabs, a significant and reproducible decrease in SDF-1-induced U937 cells migration was observed : 50% with 414H5 Mab and more than 80% with 515H7 Mab.

Example 11: Anti-CXCR4 Mab 414H5 inhibition of MDA-MB-231 xenograft tumor growth in Nod/Scid mice

The goal of these experiments was to evaluate the ability of anti-CXCR4 Mabs 414H5 and 515H7 to inhibit the growth of MDB-MB-231 xenograft in Nod/Scid mice.

MDA-MB-231 cells from ECACC were routinely cultured in DMEM medium (Invitrogen Corporation, Scotland, UK), 10% FCS (Sigma, St Louis MD, USA). Cells were split 48 hours before engraftment so that they were in exponential phase of

growth. Ten million MDA-MB-231 cells were engrafted in PBS to 7 weeks old Nod/Scid mice (Charles River, France). Five days after implantation, tumors were measurable (34 mm^3<V^3<40 mm^3) and animals were divided into groups of 6 mice with comparable tumor size. Mice were treated i.p. with a 2 mg/mouse loading dose of Mab 414H5 and Mab 515H7, respectively.

Then, mice were injected twice a week at 1 mg/dose/mouse of Mab 414H5 twice a week or 0.5 mg/dose/mouse of Mab 515H7 three time a week. A PBS group was introduced as a control group in this experiment. Tumor volume was measured twice a week and calculated by the formula: $\pi/6$ X length X width X height. Statistical analysis were performed at each measure using a Mann-Whitney test.

In these experiments, no mortality was observed during treatment. Compared to PBS group, there was a significant inhibition of tumor growth between D7 and D39 ($p \leq 0.002$) for 415H5 Mab 1mg/dose or 515H7 0.5mg/dose and the average tumor volume after 5 weeks of treatment was reduced by 82% and 50% versus PBS for Mab 415H5 and 515H7, respectively (Figures 11A and 11B).

Example 12: Anti-CXCR4 Mab 414H5 activity in U937 mice survival model

U937 cells from ATCC were cultured in RPMI 1640 medium, 10% FCS, 1% L-Glutamine. Cells were split two days before engraftment so that they were in exponential phase of growth. Ten million U937 cells injected i.p. to female NOD/SCID mice. Two days after implantation, mice were treated s.c. with a loading dose of 2 mg of 414H5 mAb/mouse and then twice a week with 1 mg of antibody/mouse. Control mice received PBS injections as it has been shown in previous studies that no difference in survival was observed between mice injected with PBS and mice administered with a mouse IgG isotype control. Mice survival was monitored every day.

Results described in figure 12 showed that mice treated with the 414H5 Mab had a dramatic and significant increase in life span with T/C% about 343.

Example 13: CXCR4 receptor-mediated mobilization of intracellular calcium stores

This functional assay was designed to monitor CXCR4 receptor signaling via stimulation of the phospholipase C pathway, inducing calcium liberation from intracellular stores from the endoplasmic reticulum.

CHO-K1 cells stably and constitutively expressing wild-type CXCR4 receptor were obtained as described in the example above. MDA-MB-231 (human breast adenocarcinoma) and U937 (human lymphoma) cells were propagated in complete culture medium, respectively [DMEM supplemented with 10% FCS] and [RPMI 1640 supplemented with 10% FCS, 20 mM HEPES, 1% non-essential amino acid solution, 1% sodium pyruvate, 1% L-glutamine, 4.5 g/l glucose]. All cell types were plated in black 96MW plates at a density of 100,000 cells/well in appropriate culture medium. Cells were starved overnight before conducting the experiments. Cells are loaded with the fluorescent calcium dye (Fluo-4 No Wash, Invitrogen US) in loading buffer [HBSS 1x, HEPES 20 mM, Probenicid acid 25 mM] for 30 min. at 37 °C followed by 30 min. at 25°C. Stimulation by SDF-1 was performed by direct injection into each well. For antagonism experiments, 10 µl of Mab solution are added directly into the loading buffer at least 10 min. before SDF-1. Kinetic fluorescence measurements are performed on a multi-mode fluorescence microplate reader Mithras LB940 (Berthold) using the following settings: excitation at 485 nm, emission at 535 nm, excitation energy at 10000 arbitrary units. Fluorescence in each well is recorded during 0,1 second every second and for a time period of 20 sec prior SDF-1 injection (basal signal). Then 20 µl of SDF-1 are injected and data recording follows for a time period of 2 min. Each experimental condition is performed in duplicate. Values for each well are first corrected by substracting the basal fluorescence and the fluorescence emitted by a control well without cells. Relative data are expressed as a percentage of the maximal stimulation obtained by SDF-1 (100 nM).

SDF1 (100 nM) induced a rapid and strong release of intracellular calcium in recombinant CHO/CXCR4, whereas no fluorescence signal was detected in naïve CHO-K1 cells. The maximal intensity reached > 160 % over basal fluorescence and was observed at about 30 sec. upon stimulation by SDF-1; similar kinetic curves were observed with both MDA-MB-231 and U-937 (Figures 13A, 13B, 13C), although the maximal fluorescence intensity by SDF-1 (100 nM) was lower (130-140 % over basal).

515H7 antibody (133 nM) yielded a strong and almost complete inhibition of the SDF-1 (100 nM)-induced calcium signal in all three investigated cell lines.

Example 14: Anti-CXCR4 Mab 414H5 inhibition of T-cell KARPAS 299 xenograft tumor growth in Nod/Scid mice

The goal of this experiment was to evaluate the ability of anti-CXCR4 Mab 414H5 to inhibit the growth of KARPAS 299 xenograft in Nod/Scid mice.

KARPAS 299 cells from ECACC were routinely cultured in RPMI medium, 1% L-Glu and 10% FCS (Sigma, St Louis MD, USA). Cells were split 48 hours before engraftment so that they were in exponential phase of growth. Five million KARPAS 299 cells were engrafted in PBS to 7 weeks old Nod/Scid mice (Charles River, France). Five days after implantation, tumors were measurable (32 $mm^3<V^3<49$ mm^3) and animals were divided into groups of 6 mice with comparable tumor size. Mice were treated i.p. with a 2 mg/mouse loading dose of Mab 414H5.

Then, mice were injected twice a week at 1 mg/dose/mouse of Mab 414H5. A PBS group was introduced as a control group in this experiment. Tumor volume was measured twice a week and calculated by the formula: $\pi/6$ X length X width X height. Statistical analysis were performed at each measure using a Mann-Whitney test.

In this experiment, no mortality was observed during treatment. Compared to PBS group, there was a significant inhibition of tumor growth between D7 and D33 ($p \leq 0.002$) for 414H5 Mab 1mg/dose and the average tumor volume after 5 weeks of treatment was reduced by 73 % for Mab 414H5 versus PBS (Figure 14).

Example 15: Anti-CXCR4 Mab 515H7 activity in U937 mice survival model

U937 cells from ATCC were cultured in RPMI 1640 medium, 10% FCS, 1% L-Glutamine. Cells were split two days before engraftment so that they were in exponential phase of growth. Ten million U937 cells injected i.p. to female NOD/SCID mice. Two days after implantation, mice were treated s.c. with a loading dose of 2 mg of 515H7 Mab/mouse and then twice a week with 1 mg of antibody/mouse. Control mice received PBS injections as it has been shown in previous studies that no difference in survival was observed between mice injected with PBS and mice administered with a mouse IgG isotype control. Mice survival was monitored every day.

Results described in figure 15 showed that mice treated with 515H7 Mab had a dramatic and significant increase in life span with T/C% about 280 for 515H7 Mab (Figure 15).

Example 16: Anti-CXCR4 Mab 515H7 inhibition of T-cell KARPAS 299 xenograft tumor growth in Nod/Scid mice

The goal of this experiment was to evaluate the ability of anti-CXCR4 Mab 515H7 to inhibit the growth of KARPAS 299 xenograft in Nod/Scid mice.

KARPAS 299 cells from ECACC were routinely cultured in RPMI medium, 1% L-Glu and 10% FCS (Sigma, St Louis MD, USA). Cells were split 48 hours before engraftment so that they were in exponential phase of growth. Five million KARPAS 299 cells were engrafted in PBS to 7 weeks old Nod/Scid mice (Charles River, France). Five days after implantation, tumors were measurable (32 $mm^3<V^3<49$ mm^3) and animals were divided into groups of 6 mice with comparable tumor size. Mice were treated i.p. with a 2 mg/mouse loading dose of Mab 515H7.

Then, mice were injected twice a week at 1 mg/dose/mouse of Mab 515H7. A PBS group was introduced as a control group in this experiment. Tumor volume was measured twice a week and calculated by the formula: $\pi/6$ X length X width X height. Statistical analysis were performed at each measure using a Mann-Whitney test.

In these experiments, no mortality was observed during treatment. Compared to PBS group, there was a significant inhibition of tumor growth between D7 and D33 ($p \leq 0.002$) for 515H7 Mab 1mg/dose and the average tumor volume after 5 weeks of treatment was reduced by 63% for Mab 515H7 versus PBS (Figure 16).

Example 17: Production of anti-CXCR4 chimeric Mabs c414H5 and c515H7

Chimeric formats of murine 414H5 and 515H7 Mabs were designed: they correspond to the light and heavy chain variable domains of the murine antibodies of interest, genetically fused to human Ckappa and IgG1 constant domains. All recombinant Mabs were produced upon transient transfection by using the HEK293/EBNA system with a pCEP4 expression vector (InVitrogen, US).

The entire nucleotide sequences corresponding to the variable domains of 414H5 and 515H7 Mabs light and heavy chains were synthesized by global gene synthesis (Genecust, Luxembourg). They were subcloned into a pCEP4 vector (InVitrogen, US) carrying the entire coding sequence of the constant domain of either the light [Ckappa] or the heavy [CH1-Hinge-CH2-CH3] chain of a human IgG1 immunoglobulin. All cloning steps were performed according to conventional molecular biology techniques as described in the Laboratory manual (Sambrook and Russel, 2001) or according to the supplier's instructions. Each genetic construct was fully validated by nucleotide sequencing using Big Dye terminator cycle sequencing kit (Applied Biosystems, US) and analyzed using a 3100 Genetic Analyzer (Applied Biosystems, US).

Suspension-adapted HEK293 EBNA cells (InVitrogen, US) were routinely grown in 250 ml flasks in 50 ml of serum-free medium Excell 293 (SAFC Biosciences) supplemented with 6 mM glutamine on an orbital shaker (110 rpm rotation speed). Transient transfection was performed with 2.106 cells/ml using linear 25 kDa polyethyleneimine (PEI) (Polysciences) prepared in water at a final concentration of 1 mg/ml mixed and plasmid DNA (final concentration of 1.25 µg/ml for heavy to light chain plasmid ratio of 1:1). At 4 hours post-transfection, the culture was diluted with one volume of fresh culture medium to achieve a final cell density of 106 cells/ml. Cultivation process was monitored on the basis of cell viability and Mab production. Typically, cultures were maintained for 4 to 5 days. Mabs were purified using a conventional chromatography approach on a Protein A resin (GE Healthcare, US). All different Mabs were produced at levels suitable with functional evaluations. Productivity levels are typically ranging between 6 and 15 mg/l of purified Mabs.

Example 18: Characterization by FACS analysis of anti-CXCR4 chimeric Mabs c414H5 and c515H7 binding specificity and cancer cell line recognition

In this experiment, specific binding to human CXCR4 of anti-CXCR4 chimeric Mabs c414H5 and c515H7 was examined by FACS analysis.

NIH3T3, NIH3T3-hCXCR4 transfected cells and MDA-MB-231 cancer cell line were incubated with 10 µg/mL of monoclonal antibody c414H5 and c515H7. The cells were then washed with 1%BSA/PBS/0.01% NaN3. Next, Alexa-labeled secondary antibodies were added to the cells and were allowed to incubate at 4°C for 20 min. The

cells were then washed again two times. Following the second wash, FACS analysis was performed. Results of these binding studies are provided in the following Table 5 which shows [Mean Fluorescence Intensity (MFI) obtained by FACS] that anti-CXCR4 chimeric Mabs c414H5 and c515H7 bound specifically to human CXCR4-NIH3T3 transfected cell line and also recognize human cancer cell lines, for example MDA-MB-231 breast cancer cells.

Table 5

Clone (10 µg/ml)	MFI on cell lines	
	NIH3T3-CXCR4	MDA-MB-231
c414H5	1039	Not tested
c515H7	2294	118

Example 19: Competition binding of anti-CXCR4 murine Mabs m414H5 and m515H7 and chimeric Mabs c414H5 and c515H7 for [^{125}I]SDF-1 at CHO-K1 membranes stably expressing human CXCR4 receptor

This assay allows to evaluate the ability of murine Mabs m414H5, m515H7 and chimeric Mabs c414H5, c515H7 to compete for binding of radio labeled [^{125}I]SDF-1 to human CXCR4 receptor, at either orthosteric or allosteric binding sites.

CHO-K1 cells, stably and constitutively expressing human CXCR4 receptor were obtained upon transfection of naïve CHO-K1 cells (ATCC CCL-61) with a mammalian expression vector carrying the whole coding sequence of human CXCR4 receptor (RefSeq NM_003467). Cells were propagated in complete culture medium [DMEM-Ham's F12 supplemented with 5 % fetal calf serum (FCS) and 500 µg/ml of geneticin]. Radioligand binding experiments were conducted on cell membranes obtained upon mechanical scrapping of CHO/CXCR4 cells in lysis buffer [Hepes 20mM, pH 7.4, NaCl 150mM] followed by centrifugation (10000g, 15 min). [^{125}I]SDF-1 binding (specific activity: 1500 Ci/mmol) was performed using the SPA technology (scintillation proximity assay – GE Healthcare). Briefly, cell membranes (30 µg/well) were incubated in binding buffer [Hepes 20mM, pH 7.4, $CaCl_2$ 1mM, $MgCl_2$ 5mM, NaCl 150mM, BSA 1%] together with compound to evaluate (SDF-1 or mAb), radioligand (1 nM) and finally SPA-WGA-PVT beads (7.3 mg/well). Binding equilibrium was reach after 1H at 25°C. Upon centrifugation [1000 g for 10 min.]

radioactive counts were measured in a scintillation counter (TopCount, Perkin Elmer). Non-specific (NS) binding was estimated in the presence of 10 μM of unlabelled SDF-1.

Anti-CXCR4 Mabs (100 nM) efficiently competed for [^{125}I]SDF-1 binding with the following rank order of competition efficacy (% inhibition of [^{125}I]SDF-1): m515H7 (62±10%), c515H7 (55±4%), m414H5 (30±5%) and c414H5 (21±10 %) (Figure 17).

Example 20: Modulation of [^{35}S]GTPγS binding at cellular membranes expressing wild type CXCR4 receptor by anti-CXCR4 murine Mabs m414H5 and m515H7 and chimeric Mabs c414H5 and c515H7

This functional assay allows to monitor G protein activation via wild type human CXCR4 receptor and its modulation by anti-CXCR4 murine Mabs m414H5, m515H7 and chimeric Mabs c414H5, c515H7.

NIH-3T3 cells stably and constitutively expressing wild-type CXCR4 receptor were obtained as described in the example above for CHO-K1 cells. HeLa (human cervix carcinoma) cells were propagated in complete culture medium [EMEM supplemented with 10% FCS, 1% L-glutamine, 2 μM sodium bicarbonate]. [^{35}S]GTPγS binding was performed on cellular membranes obtained upon mechanical scrapping in lysis buffer [Hepes 20mM, pH 7.4, NaCl 150mM] and further centrifugation (10000 g, 15 min). Incorporation and detection of [^{35}S]GTPγS (specific activity: 1000 Ci/mmol) was performed using the SPA technology (scintillation proximity assay - GE Healthcare). Briefly, cell membranes (10 μg/well) were incubated in binding buffer [Hepes 20mM, GDP 3μM, $MgCl_2$ 10mM, NaCl 100mM, EDTA 1mM, pH=7.4] together with compound to evaluate (SDF-1 and Mab of interest), [^{35}S]GTPγS (0.2-0.4 nM) and finally SPA-WGA-PVT beads (7.3 mg/well). Binding reaction was performed during 1H at 25°C. Upon centrifugation [1000 g for 10 min.] radioactive counts were measured in a scintillation counter (TopCount, Perkin Elmer). IC_{50} were calculated for each Mab.

Under these experimental conditions, IC_{50} of m414H5, c414H5, m515H7 and c515H7 Mabs, as determined in NIH3T3/CXCR4 cells were 1.6 nM, 1.1 nM, 1.9 nM and 1.5 nM, respectively (Figure 18). The IC_{50} of m414H5, c414H5, m515H7 and

c515H7 Mabs determined using Hela cells in the same experimental conditions were 0.5 nM, 0.3 nM, 0.2 nM and 0.6 nM, respectively (Figure 19).

Example 21: Association of CXCR4 with different interaction partners: homo and heterodimerization, recruitment of β-arrestin via a bioluminescence resonance energy transfer (BRET) approach and effect of murine Mabs m414H5, m515H7 and chimeric Mabs c414H5 and c515H7 on these dimmers

This functional assay allows to evaluate the conformational changes induced upon SDF-1 and/or m414H5, m515H7 murine Mabs and c414H5, and c515H7 chimeric Mabs binding to CXCR4 receptor at the level of CXCR4 homo-dimer and CXCR2/CXCR4 hetero-dimer formation as well as the recruitment of the β-arrestin-2 signaling protein.

Expression vectors for each of the investigated interaction partners were constructed as fusion proteins with the corresponding dye (*Renilla reniformis* luciferase, Rluc and Yellow fluorescent protein, YFP) by applying conventional molecular biology techniques. Two days prior performing BRET experiments, HEK293 cells were transiently transfected with expression vectors coding for the corresponding BRET partners: [CXCR4/Rluc + CXCR4/YFP] to study CXCR4 homo dimerization, [CXCR4-Rluc + CXCR2-YFP] to study CXCR4 and CXCR2 hetero-dimerization and [CXCR4-Rluc + β-arr2-YFP] to study CXCR4-mediated recruitment of β-arrestin-2. The day after, cells were distributed in poly-lysine pre-coated white 96 MW plates in complete culture medium [DMEM supplemented with 10 % FBS]. Cells were first cultivated at 37°C with CO_2 5 % in order to allow cell attachment to the plate. Cells were then starved with 200 µl DMEM/well overnight. Immediately prior to the BRET experiment, DMEM was removed and cells were quickly washed with PBS. Cells were then incubated in PBS in the presence or absence of antibody, 15 min at 37°C prior to the addition of coelenterazine H 5 µM with or without SDF-1 100 nM in a final volume of 50 µl. After incubation for 5 minutes at 37°C and further incubation for 20 min at room temperature only for homo and hetero-dimers, light-emission acquisition at 485 nm and 530 nm was initiated using the Mithras LB940 multilabel reader (Berthold) (1s/wavelength/well repeated 15 times at room temperature).

Calculation of BRET ratio was performed as previously described (Angers et al., 2000): [(emission$_{530\ nm}$) - (emission$_{485\ nm}$) X Cf] / (emission$_{485\ nm}$), where Cf = (emission$_{530\ nm}$) / (emission$_{485\ nm}$) for cells expressing the Rluc fusion protein alone under the same experimental conditions. Simplifying this equation shows that BRET ratio corresponds to the ratio 530/485 nm obtained when the two BRET partners are present, corrected by the ratio 530/485 nm obtained under the same experimental conditions, when only the partner fused to Rluc is present in the assay. For sake of readability, results are expressed in milliBRET units (mBU); mBU corresponds to the BRET ratio multiplied by 1000.

SDF1 (100 nM) increased by about 10 % the BRET signal resulting from the spatial proximity of the donor and acceptor proteins fused to CXCR4 receptor, it is likely to indicate CXCR4/CXCR4 homo-dimers formation or conformational changes of pre-existing dimers (Figure 20A). Interestingly, SDF1 (100 nM) decreased by about 17 % the BRET signal resulting from the spatial proximity of the donor and acceptor proteins fused to CXCR4 and CXCR2, likely indicating as well CXCR2/CXCR4 hetero-dimers formation or conformational changes of pre-existing dimers (Figure 20B). In this latter case, SDF-1-activated conformation of CXCR4/CXCR2 seems less favorable for BRET energy transfer. In both cases, m414H5, c414H5 and m515H7, c515H7 Mabs were able to modulate SDF-1-induced conformational changes for CXCR4 homo-dimers (75 % inhibition of SDF-1-induced BRET increase for c414H5 and 96% inhibition of SDF-1-induced BRET increase for c515H7, Figure 20A) as well as for CXCR2/CXCR4 hetero-dimer formation (77 % inhibition of SDF-1-induced BRET decrease for c414H5 and 98 % inhibition of SDF-1-induced BRET decrease for c515H7, Figure 20B). m414H5, c414H5, m515H7 and c515H7 Mabs were also able to modulate by themselves CXCR4/CXCR4 and CXCR2/CXCR4 spatial proximity respectively, indicating an influence of these Mabs on both CXCR4/CXCR4 homo and CXCR2/CXCR4 hetero-dimer conformation (Figures 20A and 20B).

CXCR4 activation by SDF-1 (100 nM) yielded a strong recruitment of the intracellular signaling molecule β-arrestin, as shown by the 400 % enhancement in BRET signal (Figure 20C). This recruitment was partially inhibited by c414H5 and by c515H7 Mabs (about 63 % inhibition for c414H5 and 93% for c515H7, Figure 20C) showing the effect of these Mabs on signaling.

Example 22: CXCR4 receptor-mediated mobilization of intracellular calcium stores

This functional assay was designed to monitor CXCR4 receptor signaling via stimulation of the phospholipase C pathway, inducing calcium liberation from intracellular stores from the endoplasmic reticulum.

CHO-K1 cells stably and constitutively expressing wild-type CXCR4 receptor were obtained as described in the example above. U937 (human lymphoma) cells were propagated in complete culture medium, respectively [DMEM supplemented with 10% FCS] and [RPMI 1640 supplemented with 10% FCS, 20 mM HEPES, 1% non-essential amino acid solution, 1% sodium pyruvate, 1% L-glutamine, 4.5 g/l glucose]. All cell types were plated in black 96MW plates at a density of 100,000 cells/well in appropriate culture medium. Cells were starved overnight before conducting the experiments. Cells are loaded with the fluorescent calcium dye (Fluo-4 No Wash, Invitrogen US) in loading buffer [HBSS 1x, HEPES 20 mM, Probenicid acid 25 mM] for 30 min. at 37 °C followed by 30 min. at 25°C. Stimulation by SDF-1 was performed by direct injection into each well. For antagonism experiments, 10 µl of Mab solution are added directly into the loading buffer at least 10 min. before SDF-1. Kinetic fluorescence measurements are performed on a multi-mode fluorescence microplate reader Mithras LB940 (Berthold) using the following settings: excitation at 485 nm, emission at 535 nm, excitation energy at 10000 arbitrary units. Fluorescence in each well is recorded during 0.1 second every second and for a time period of 20 sec prior SDF-1 injection (basal signal). Then 20 µl of SDF-1 are injected and data recording follows for a time period of 2 min. Each experimental condition is performed in duplicate. Values for each well are first corrected by substracting the basal fluorescence and the fluorescence emitted by a control well without cells. Relative data are expressed as a percentage of the maximal stimulation obtained by SDF-1 (100 nM).

SDF1 (100 nM) induced a rapid and strong release of intracellular calcium in recombinant CHO/CXCR4, whereas no fluorescence signal was detected in naïve CHO-K1 cells. The maximal intensity reached > 140 % over basal fluorescence and was observed at about 40 sec. upon stimulation by SDF-1; similar kinetic curves were observed with U-937 cells (Figures 21A, 21B). Chimeric antibody c515H7 (133 nM)

yielded a strong and almost complete inhibition of the SDF-1 (100 nM)-induced calcium signal in both investigated cell lines.

Example 23: Effect of anti-CXCR4 murine Mabs m414H5, m515H7 and chimeric Mabs c414H5, c515H7 on SDF-1-induced U937 cells migration

To evaluate the inhibiting effect of the anti-CXCR4 Mabs m414H5, m515H7, c414H5 and c515H7 on the migration process, 100 000 U-937 cells in RPMI 1640 medium supplemented with 2% FCS, were plated in the upper chamber of migration chambers (24 wells plates with 8-µm pore size) either in presence or in absence of SDF-1 in the lower part of the wells and with or without Mabs c414H5, m414H5, c515H7 and m515H7 in the upper chamber. In this test murine IgG2a and IgG2b were introduced as an isotype controls. Two hours after plating, migrating cells were counted. The results presented in figures 22A (for c414H5 versus m414H5) and 22B (for c515H7 versus m515H7) demonstrated that, as expected SDF-1 was able to induce a significant increase of U-937 cells migration. No effect was observed when cells were incubated with the IgG2 isotype control. In contrast, for cells incubated with the c414H5, m414H5, c515H7 and m515H7 Mabs, a significant and reproducible decrease in SDF-1-induced U937 cells migration was observed: about 50% with c414H5 and m414H5 Mabs and more than 80% with c515H7 and m515H7 Mabs.

Example 24: Anti-CXCR4 chimeric Mabs c414H5 and c515H7 activity in U937 mice survival model

U937 cells from ATCC were cultured in RPMI 1640 medium, 10% FCS, 1% L-Glutamine. Cells were split two days before engraftment so that they were in exponential phase of growth. Ten million U937 cells injected i.p. to female NOD/SCID mice. Two days after implantation, mice were treated s.c. with a loading dose of 2 mg of c414H5 or c515H7 Mab/mouse and then twice a week with 1 mg of antibody/mouse. Control mice received PBS injections as it has been shown in previous studies that no difference in survival was observed between mice injected with PBS and mice administered with a mouse IgG isotype control. Mice survival was monitored every day.

Results described in figure 23 showed that mice treated with the c414H5 and c515H7 Mabs had a dramatic and significant increase in life span with T/C% about 210 and 180 for c414H5 and c515H7, respectively.

CLAIMS

1. Process for the selection of an anti-CXCR4 antibody, or one of its functional fragments or derivatives, capable of inhibiting activation of CXCR4, characterized in that it comprises the following steps:

i) screening the generated antibodies and selecting antibodies capable to bind specifically to CXCR4 and also to modulate CXCR4 activation;

ii) testing the selected antibodies of step i) and selecting antibodies capable to induce CXCR4 homodimers conformational change, and then

iii) testing the selected antibodies of step ii) and selecting antibodies capable to induce CXCR4/CXCR2 heterodimers conformational change.

2. Process according to claim 1, wherein step ii) consists in evaluating antibodies by BRET analysis on cells expressing both CXCR4-RLuc/CXCR4-YFP and selecting antibodies capable to inhibit at least 40 %, of the BRET signal.

3. Process according to claim 1, wherein step iii) consists in evaluating antibodies by BRET analysis on cells expressing both CXCR4-RLuc/CXCR2-YFP and selecting antibodies capable to inhibit at least 40 %, of the BRET signal.

4. An isolated antibody, or a derived compound or functional fragment of same, susceptible of being obtained by a process as claimed in claim 1, said antibody consisting of a monoclonal antibody capable of inhibiting at least 40 %, of the BRET signal on cells expressing both CXCR4-RLuc/CXCR4-YFP and/or CXCR4-RLuc/CXCR2-YFP.

5. An antibody, or a derived compound or functional fragment of same, capable of inhibiting activation of CXCR4, characterized in that the said antibody, or derived compound or functional fragment of same, is selected in the group consisting of:

a) an antibody, or a derived compound or functional fragment of same, comprising:

- a light chain comprising, according to IMGT, the CDR-L1 of the sequence SEQ ID No. 1, the CDR-L2 of the sequence SEQ ID No. 2 and the CDR-L3 of the sequence SEQ ID No. 3; and

- a heavy chain comprising, according to IMGT, the CDR-H1 of the sequence SEQ ID No. 7, the CDR-H2 of the sequence SEQ ID No. 5 and the CDR-H3 of the sequence SEQ ID No. 8;

b) an antibody, or a derived compound or functional fragment of same, comprising:

- a light chain comprising, according to Kabat, the CDR-L1 of the sequence SEQ ID No. 9, the CDR-L2 of the sequence SEQ ID No. 10 and the CDR-L3 of the sequence SEQ ID No. 3; and

- a heavy chain comprising, according to Kabat, the CDR-H1 of the sequence SEQ ID No. 11, the CDR-H2 of the sequence SEQ ID No. 12 and the CDR-H3 of the sequence SEQ ID No. 6;

c) an antibody, or a derived compound or functional fragment of same, comprising:

- a light chain comprising, according to IMGT, the CDR-L1 of the sequence SEQ ID No. 40, the CDR-L2 of the sequence SEQ ID No. 2 and the CDR-L3 of the sequence SEQ ID No. 41; and

- a heavy chain comprising, according to IMGT, the CDR-H1 of the sequence SEQ ID No. 44, the CDR-H2 of the sequence SEQ ID No. 5 and the CDR-H3 of the sequence SEQ ID No. 45; and

d) an antibody, or a derived compound or functional fragment of same, comprising:

- a light chain comprising, according to Kabat, the CDR-L1 of the sequence SEQ ID No. 46, the CDR-L2 of the sequence SEQ ID No. 47 and the CDR-L3 of the sequence SEQ ID No. 41; and

- a heavy chain comprising, according to Kabat, the CDR-H1 of the sequence SEQ ID No. 48, the CDR-H2 of the sequence SEQ ID No. 49 and the CDR-H3 of the sequence SEQ ID No. 43.

6. An antibody, or a derived compound or functional fragment of same, according to claim 5, characterized in that the said antibody, or derived compound or functional fragment of same, is selected in the group consisting of:

a) an antibody, or a derived compound or functional fragment of same, comprising a light chain sequence comprising the amino acid sequence SEQ ID No. 13, and in that it comprises a heavy chain sequence comprising the amino acid sequence SEQ ID No. 14; and

b) an antibody, or a derived compound or functional fragment of same, characterized in that it comprises a light chain sequence comprising the amino acid sequence SEQ ID No. 50, and in that it comprises a heavy chain sequence comprising the amino acid sequence SEQ ID No. 51.

7. A murine hybridoma selected from hybridoma filed at the CNCM, Pasteur Institute, Paris, on October 22, 2007, under number I-3860 or filed at the CNCM, Pasteur Institute, Paris, on June 25, 2008, under number I-4019.

8. An antibody secreted by the hybridoma according to claim 7.

9. An antibody, or a derived compound or functional fragment of same, according to claims 4 to 6 and 8 characterized in that the said antibody, or derived compound or functional fragment of same, consists of a chimeric antibody.

10. An antibody, or a derived compound or functional fragment of same, according to claim 9, characterized in that the said antibody, or derived compound or functional fragment of same, is selected in the group consisting of:

a) a chimeric antibody comprising a light chain sequence comprising the amino acid sequence SEQ ID No. 64, and in that it comprises a heavy chain sequence comprising the amino acid sequence SEQ ID No. 65; and

b) a chimeric antibody, or a derived compound or functional fragment of same, comprising a light chain sequence comprising the amino acid sequence SEQ ID No. 66, and in that it comprises a heavy chain sequence comprising the amino acid sequence SEQ ID No. 67.

11. An isolated nucleic acid characterized in that the said isolated nucleic acid is selected among the following nucleic acids:

- a nucleic acid, DNA or RNA, coding for an antibody, or for a derived compound or functional fragment of same, according to one of the claims 4 to 6 and 8 to 10;

- a nucleic acid complementary to a nucleic acid as defined in a); and

- a nucleic acid of at least 18 nucleotides capable of hybridizing under highly stringent conditions with at least one of the CDRs of nucleic acid sequences SEQ ID Nos. 15 to 26 or SEQ ID Nos. 52 to 61.

12. A vector composed of a nucleic acid according to claim 11.

13. A host cell comprising a vector according to claim 12.

14. A transgenic animal, except for man, comprising a cell transformed by a vector according to claim 13.

15. A method for producing an antibody, or a derived compound or functional fragment of same, according to one of the claims 4 to 6 and 8 to 10, characterized in that said method comprises the following steps:

- the culture in a medium of and the suitable culture conditions for a host cell according to claim 13; and

- the recovery of said antibody, or one of its functional fragments, thus produced from the culture medium or from said cultured cells.

16. An antibody, or a derived compound or functional fragment of same, according to one of the claims 4 to 6 and 8 to 10, for use as a drug.

17. A pharmaceutical composition comprising as an active ingredient a compound consisting of an antibody, or a derived compound or functional fragment of same, according to one of the claims 4 to 6, 9 to 10 and 16.

18. A pharmaceutical composition according to claim 17, characterized in that it comprises, in addition, as a combination product for use in a simultaneous, separated or extended fashion, an anti-tumor antibody other that an antibody directed against CXCR4.

19. A pharmaceutical composition according to claims 17 or 18, characterized in that it comprises, in addition, as a combination or conjugation product for use in a simultaneous, separated or extended fashion, a cytotoxic/cytostatic agent, a cellular toxin and/or a radioisotope.

20. A pharmaceutical composition according to one of the claims 17 to 18, for use as a drug.

21. An antibody, or a derived compound or functional fragment of same, according to one of the claims 4 to 6, 8 to 10 and 16, and/or of a pharmaceutical composition according to any of the claims 17 to 20, as a drug for modulating CXCR4 activity in a cell.

22. Use of an antibody, or a derived compound or functional fragment of same, according to one of the claims 4 to 6, 8 to 10 and 16, and/or of a pharmaceutical composition according to any of the claims 17 to 20, in the manufacture of a medicament for prevention or treatment of cancer.

23. Use according to claim 22, characterized in that said cancer is a cancer selected

among prostate cancer, osteosarcoma, lung cancer, breast cancer, endometrial cancer, multiple myeloma, ovarian cancer, pancreatic cancer and colon cancer.

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Cys Lys Ala Val His Val Ile Tyr Thr Val Asn Leu Tyr Ser Ser Val
        115                 120                 125

Leu Ile Leu Ala Phe Ile Ser Leu Asp Arg Tyr Leu Ala Ile Val His
    130                 135                 140
```

```
Ala Thr Asn Ser Gln Arg Pro Arg Lys Leu Leu Ala Glu Lys Val Val
145                 150                 155                 160

Tyr Val Gly Val Trp Ile Pro Ala Leu Leu Leu Thr Ile Pro Asp Phe
                165                 170                 175

Ile Phe Ala Asn Val Ser Glu Ala Asp Asp Arg Tyr Ile Cys Asp Arg
            180                 185                 190

Phe Tyr Pro Asn Asp Leu Trp Val Val Val Phe Gln Phe Gln His Ile
        195                 200                 205

Met Val Gly Leu Ile Leu Pro Gly Ile Val Ile Leu Ser Cys Tyr Cys
    210                 215                 220

Ile Ile Ile Ser Lys Leu Ser His Ser Lys Gly His Gln Lys Arg Lys
225                 230                 235                 240

Ala Leu Lys Thr Thr Val Ile Leu Ile Leu Ala Phe Phe Ala Cys Trp
                245                 250                 255

Leu Pro Tyr Tyr Ile Gly Ile Ser Ile Asp Ser Phe Ile Leu Leu Glu
            260                 265                 270

Ile Ile Lys Gln Gly Cys Glu Phe Glu Asn Thr Val His Lys Trp Ile
        275                 280                 285

Ser Ile Thr Glu Ala Leu Ala Phe Phe His Cys Cys Leu Asn Pro Ile
    290                 295                 300

Leu Tyr Ala Phe Leu Gly Ala Lys Phe Lys Thr Ser Ala Gln His Ala
305                 310                 315                 320

Leu Thr Ser Val Ser Arg Gly Ser Ser Leu Lys Ile Leu Ser Lys Gly
                325                 330                 335

Lys Arg Gly Gly His Ser Ser Val Ser Thr Glu Ser Glu Ser Ser Ser
            340                 345                 350

Phe His Ser Ser
        355


<210>  31
<211>  360
<212>  PRT
<213>  Mus musculus

<400>  31

Met Glu Asp Phe Asn Met Glu Ser Asp Ser Phe Glu Asp Phe Trp Lys
1                   5                   10                  15

Gly Glu Asp Leu Ser Asn Tyr Ser Tyr Ser Ser Thr Leu Pro Pro Phe
            20                  25                  30

Leu Leu Asp Ala Ala Pro Cys Glu Pro Glu Ser Leu Glu Ile Asn Lys
        35                  40                  45

Tyr Phe Val Val Ile Ile Tyr Ala Leu Val Phe Leu Leu Ser Leu Leu
    50                  55                  60

Gly Asn Ser Leu Val Met Leu Val Ile Leu Tyr Ser Arg Val Gly Arg
65                  70                  75                  80

Ser Val Thr Asp Val Tyr Leu Leu Asn Leu Ala Leu Ala Asp Leu Leu
                85                  90                  95
```

```
Phe Ala Leu Thr Leu Pro Ile Trp Ala Ala Ser Lys Val Asn Gly Trp
            100                 105                 110

Ile Phe Gly Thr Phe Leu Cys Lys Val Val Ser Leu Leu Lys Glu Val
        115                 120                 125

Asn Phe Tyr Ser Gly Ile Leu Leu Leu Ala Cys Ile Ser Val Asp Arg
    130                 135                 140

Tyr Leu Ala Ile Val His Ala Thr Arg Thr Leu Thr Gln Lys Arg Tyr
145                 150                 155                 160

Leu Val Lys Phe Ile Cys Leu Ser Ile Trp Gly Leu Ser Leu Leu Leu
                165                 170                 175

Ala Leu Pro Val Leu Leu Phe Arg Arg Thr Val Tyr Ser Ser Asn Val
            180                 185                 190

Ser Pro Ala Cys Tyr Glu Asp Met Gly Asn Asn Thr Ala Asn Trp Arg
        195                 200                 205

Met Leu Leu Arg Ile Leu Pro Gln Ser Phe Gly Phe Ile Val Pro Leu
    210                 215                 220

Leu Ile Met Leu Phe Cys Tyr Gly Phe Thr Leu Arg Thr Leu Phe Lys
225                 230                 235                 240

Ala His Met Gly Gln Lys His Arg Ala Met Arg Val Ile Phe Ala Val
                245                 250                 255

Val Leu Ile Phe Leu Leu Cys Trp Leu Pro Tyr Asn Leu Val Leu Leu
            260                 265                 270

Ala Asp Thr Leu Met Arg Thr Gln Val Ile Gln Glu Thr Cys Glu Arg
        275                 280                 285

Arg Asn His Ile Asp Arg Ala Leu Asp Ala Thr Glu Ile Leu Gly Ile
    290                 295                 300

Leu His Ser Cys Leu Asn Pro Leu Ile Tyr Ala Phe Ile Gly Gln Lys
305                 310                 315                 320

Phe Arg His Gly Leu Leu Lys Ile Leu Ala Ile His Gly Leu Ile Ser
                325                 330                 335

Lys Asp Ser Leu Pro Lys Asp Ser Arg Pro Ser Phe Val Gly Ser Ser
            340                 345                 350

Ser Gly His Thr Ser Thr Thr Leu
        355                 360
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<210> 32
<211> 24
<212> DNA
<213> Mus musculus

<400> 32
gaaactctgc attctcgctt cctg 24

<210> 33
<211> 22
<212> DNA
<213> Mus musculus

<400> 33

aggactcgtt tgtacccgtt ga 22

<210> 34
<211> 26
<212> DNA
<213> Mus musculus

<400> 34
tgcagattgg ctacccaact gttgca 26

<210> 35
<211> 22
<212> DNA
<213> Mus musculus

<400> 35
ctccttcatc ctcctggaaa tc 22

<210> 36
<211> 22
<212> DNA
<213> Mus musculus

<400> 36
ccaaggaaag catagaggat gg 22

<210> 37
<211> 22
<212> DNA
<213> Mus musculus

<400> 37
gtggtcatta tctatgccct gg 22

<210> 38
<211> 22
<212> DNA
<213> Mus musculus

<400> 38
cgaccctgct gtataagatg ac 22

<210> 39
<211> 25
<212> DNA
<213> Mus musculus

<400> 39
tattcctgct gagcctgctg ggaaa 25

<210> 40
<211> 12
<212> PRT
<213> Mus musculus

<400> 40

Gln Ser Leu Phe Asn Ser Arg Thr Arg Lys Asn Tyr
1 5 10

<210> 41
<211> 8
<212> PRT
<213> Mus musculus

<400> 41

Met Gln Ser Phe Asn Leu Arg Thr
1 5

<210> 42
<211> 3
<212> PRT
<213> Mus musculus

<400> 42

Asp Asn Tyr
1

<210> 43
<211> 9
<212> PRT
<213> Mus musculus

<400> 43

Asp Val Gly Ser Asn Tyr Phe Asp Tyr
1 5

<210> 44
<211> 8
<212> PRT
<213> Mus musculus

<400> 44

Gly Phe Thr Phe Thr Asp Asn Tyr
1 5

<210> 45
<211> 11
<212> PRT
<213> Mus musculus

<400> 45

Ala Arg Asp Val Gly Ser Asn Tyr Phe Asp Tyr
1 5 10

<210> 46
<211> 17
<212> PRT
<213> Mus musculus

<400> 46

Lys Ser Ser Gln Ser Leu Phe Asn Ser Arg Thr Arg Lys Asn Tyr Leu
1 5 10 15

Ala

<210> 47
<211> 7
<212> PRT
<213> Mus musculus

<400> 47

Trp Ala Ser Ala Arg Asp Ser
1 5

<210> 48
<211> 5
<212> PRT
<213> Mus musculus

<400> 48

Asp Asn Tyr Met Ser
1 5

<210> 49
<211> 19
<212> PRT
<213> Mus musculus

<400> 49

Phe Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr Asp Tyr Ser Ala Ser
1 5 10 15

Val Arg Gly

<210> 50
<211> 112
<212> PRT
<213> Mus musculus

<400> 50

Asp Ile Val Met Ser Gln Ser Pro Ser Ser Leu Ala Val Ser Ala Gly
1 5 10 15

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Phe Asn Ser
20 25 30

Arg Thr Arg Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Ala Arg Asp Ser Gly Val
50 55 60

Pro Ala Arg Phe Thr Gly Ser Gly Ser Glu Thr Tyr Phe Thr Leu Thr
65 70 75 80

Ile Ser Arg Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Met Gln
85 90 95

Ser Phe Asn Leu Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 51
<211> 120

<212> PRT
<213> Mus musculus

<400> 51

Glu Val Asn Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Phe Thr Phe Thr Asp Asn
20 25 30

Tyr Met Ser Trp Val Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu
35 40 45

Gly Phe Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr Asp Tyr Ser Ala
50 55 60

Ser Val Arg Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gln Ser Ile
65 70 75 80

Leu Tyr Leu Gln Met Asn Ala Leu Arg Ala Glu Asp Ser Ala Thr Tyr
85 90 95

Tyr Cys Ala Arg Asp Val Gly Ser Asn Tyr Phe Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Thr Leu Thr Val Ser Ser
115 120

<210> 52
<211> 36
<212> DNA
<213> Mus musculus

<400> 52
cagagtctgt tcaacagtcg aacccgaaag aactac 36

<210> 53
<211> 24
<212> DNA
<213> Mus musculus

<400> 53
atgcaatctt ttaatcttcg gacg 24

<210> 54
<211> 9
<212> DNA
<213> Mus musculus

<400> 54
gataactac 9

<210> 55
<211> 27
<212> DNA
<213> Mus musculus

<400> 55
gatgtcggtt ccaactactt tgactac 27

<210> 56

<211> 24
<212> DNA
<213> Mus musculus

<400> 56
gggttcacct tcactgataa ctac 24

<210> 57
<211> 33
<212> DNA
<213> Mus musculus

<400> 57
gcaagagatg tcggttccaa ctactttgac tac 33

<210> 58
<211> 51
<212> DNA
<213> Mus musculus

<400> 58
aaatccagtc agagtctgtt caacagtcga acccgaaaga actacttggc t 51

<210> 59
<211> 21
<212> DNA
<213> Mus musculus

<400> 59
tgggcatccg ctagggattc t 21

<210> 60
<211> 15
<212> DNA
<213> Mus musculus

<400> 60
gataactaca tgagt 15

<210> 61
<211> 57
<212> DNA
<213> Mus musculus

<400> 61
tttattagaa acaaagctaa tggttacaca acagactaca gtgcatctgt gaggggt 57

<210> 62
<211> 336
<212> DNA
<213> Mus musculus

<400> 62
gacattgtga tgtcacagtc tccatcctcc ctggctgtgt cagcaggaga gaaggtcact 60

atgagctgca aatccagtca gagtctgttc aacagtcgaa cccgaaagaa ctacttggct 120

tggtaccagc agaagccagg gcagtctcct aaactgctga tctactgggc atccgctagg 180

gattctgggg tccctgctcg cttcacaggc agtggatctg agacatattt cactctcacc 240

atcagccgtg tgcaggctga agacctggca gtttattact gcatgcaatc ttttaatctt 300

cggacgttcg gtggaggcac caagctggaa atcaaa 336

<210> 63
<211> 360
<212> DNA
<213> Mus musculus

<400> 63
gaggtgaacc tggtggagtc tggaggaggc ttggtacagc ctgggggttc tctgagactc 60

tcctgtgcaa cttctgggtt caccttcact gataactaca tgagttgggt ccgccagcct 120

ccaggaaagg cacttgagtg gttgggcttt attagaaaca aagctaatgg ttacacaaca 180

gactacagtg catctgtgag gggtcggttc accatctcaa gagataattc ccaaagcatc 240

ctctatcttc aaatgaacgc cctgagagcc gaagacagtg ccacttatta ctgtgcaaga 300

gatgtcggtt ccaactactt tgactactgg ggccaaggca ccactctcac agtctcctca 360

<210> 64
<211> 239
<212> PRT
<213> mus musculus

<400> 64

Met Glu Ser Gln Ala Gln Val Leu Ile Leu Leu Leu Leu Trp Val Ser
1 5 10 15

Gly Thr Cys Gly Asp Ile Val Met Ser Gln Ser Pro Ser Ser Leu Ala
20 25 30

Val Ser Ala Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser
35 40 45

Leu Tyr Asn Ser Arg Thr Arg Lys Asn Tyr Leu Ala Trp Tyr Gln Gln
50 55 60

Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg
65 70 75 80

Glu Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp
85 90 95

Phe Thr Leu Thr Ile Thr Ser Val Gln Ala Glu Asp Leu Ala Val Phe
100 105 110

Tyr Cys Lys Gln Ser Tyr Asn Leu Arg Thr Phe Gly Gly Gly Thr Lys
115 120 125

Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
130 135 140

Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
145 150 155 160

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
165 170 175

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
180 185 190

Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
195 200 205

Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
210 215 220

Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 65
<211> 467
<212> PRT
<213> mus musculus

<400> 65

Met Lys Leu Trp Leu Asn Trp Val Phe Leu Val Thr Leu Leu Asn Gly
1 5 10 15

Phe Gln Cys Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Gln
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Thr Thr Ser Gly Phe Thr Phe
35 40 45

Thr Asp Tyr Tyr Met Ser Trp Val Arg Gln Ser Pro Gly Lys Ala Leu
50 55 60

Glu Trp Leu Thr Phe Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr Glu
65 70 75 80

Tyr Ser Ala Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser
85 90 95

Gln Ser Ile Leu Tyr Leu Gln Met Asn Thr Leu Arg Ala Glu Asp Ser
100 105 110

Ala Thr Tyr Tyr Cys Ala Arg Asp Ile Pro Gly Phe Ala Tyr Trp Gly
115 120 125

Gln Gly Thr Leu Val Thr Val Ser Ala Ala Ser Thr Lys Gly Pro Ser
130 135 140

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
145 150 155 160

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
165 170 175

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
180 185 190

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
195 200 205

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
210 215 220

Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys
225 230 235 240

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
245 250 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
260 265 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
275 280 285

```
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
305                 310                 315                 320

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
                325                 330                 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
            340                 345                 350

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
        355                 360                 365

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
    370                 375                 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385                 390                 395                 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                405                 410                 415

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            420                 425                 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        435                 440                 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    450                 455                 460

Pro Gly Lys
465
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<210> 66
<211> 239
<212> PRT
<213> mus musculus

<400> 66

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Met Glu Ser Gln Thr Leu Val Phe Ile Ser Ile Leu Leu Trp Leu Tyr
1               5                   10                  15

Gly Thr Cys Gly Asp Ile Val Met Ser Gln Ser Pro Ser Ser Leu Ala
            20                  25                  30

Val Ser Ala Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser
        35                  40                  45

Leu Phe Asn Ser Arg Thr Arg Lys Asn Tyr Leu Ala Trp Tyr Gln Gln
    50                  55                  60

Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Ala Arg
65                  70                  75                  80

Asp Ser Gly Val Pro Ala Arg Phe Thr Gly Ser Gly Ser Glu Thr Tyr
                85                  90                  95

Phe Thr Leu Thr Ile Ser Arg Val Gln Ala Glu Asp Leu Ala Val Tyr
            100                 105                 110

Tyr Cys Met Gln Ser Phe Asn Leu Arg Thr Phe Gly Gly Gly Thr Lys
        115                 120                 125
```

Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
130 135 140

Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
145 150 155 160

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
165 170 175

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
180 185 190

Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
195 200 205

Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
210 215 220

Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 67
<211> 469
<212> PRT
<213> mus musculus

<400> 67

Met Lys Met Trp Leu Asn Trp Val Phe Leu Val Thr Leu Leu Asn Gly
1 5 10 15

Ile Gln Cys Glu Val Asn Leu Val Glu Ser Gly Gly Gly Leu Val Gln
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Phe Thr Phe
35 40 45

Thr Asp Asn Tyr Met Ser Trp Val Arg Gln Pro Pro Gly Lys Ala Leu
50 55 60

Glu Trp Leu Gly Phe Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr Asp
65 70 75 80

Tyr Ser Ala Ser Val Arg Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser
85 90 95

Gln Ser Ile Leu Tyr Leu Gln Met Asn Ala Leu Arg Ala Glu Asp Ser
100 105 110

Ala Thr Tyr Tyr Cys Ala Arg Asp Val Gly Ser Asn Tyr Phe Asp Tyr
115 120 125

Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Ser Thr Lys Gly
130 135 140

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
145 150 155 160

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
165 170 175

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
180 185 190

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
195 200 205

```
Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
    210                 215                 220

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys
225                 230                 235                 240

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
                245                 250                 255

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
            260                 265                 270

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
        275                 280                 285

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
    290                 295                 300

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
305                 310                 315                 320

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
                325                 330                 335

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
            340                 345                 350

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
        355                 360                 365

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
    370                 375                 380

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
385                 390                 395                 400

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
                405                 410                 415

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
            420                 425                 430

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
        435                 440                 445

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
    450                 455                 460

Leu Ser Pro Gly Lys
465
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<210> 68
<211> 720
<212> DNA
<213> mus musculus

<400> 68

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atggagtcac aggcccaggt tcttatattg ctgctgctat gggtatctgg tacctgtggg     60

gacattgtga tgtcacagtc tccatcctcc ctggctgtgt cagcaggaga gaaggtcact    120

atgagctgca aatccagtca gagtctgtac aacagtagaa cccgaaagaa ctacttggct    180

tggtaccagc agaaaccagg gcagtctcct aaactgctga tctactgggc atccactagg    240

gaatctgggg tccctgatcg cttcacaggc agtggatctg ggacagattt cactctcacc    300
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atcaccagtg tgcaggctga ggacctggca gttttttact gcaagcaatc ttataatctt 360

cggacgttcg gtggaggcac caagctggaa atcaaacgta cggtggccgc tcccagcgtg 420

ttcatcttcc ccccaagcga cgagcagctg aagagcggca ccgccagcgt ggtgtgtctg 480

ctgaacaact tctaccccag ggaggccaag gtgcagtgga aggtggacaa cgccctgcag 540

agcggcaaca gccaggagag cgtcaccgag caggacagca aggactccac ctacagcctg 600

agcagcaccc tgaccctgag caaggccgac tacgagaagc acaaggtgta cgcctgtgag 660

gtgacccacc agggcctgtc agccccgtg accaagagct tcaacagggg cgagtgctga 720

<210> 69
<211> 1404
<212> DNA
<213> mus musculus

<400> 69
atgaagctgt ggctgaactg ggtgttcctg gtgaccctgc tgaacggctt ccagtgcgaa 60

gtgaaactgg tggagtctgg cggcggactg gtgcagccag gcggcagcct gagactgagc 120

tgcaccacct ccggcttcac cttcaccgac tactacatga gctgggtgcg ccagagcccc 180

ggcaaggccc tggaatggct gaccttcatc cggaacaagg ccaacggcta caccaccgag 240

tacagcgcca gcgtgaaggg ccggttcacc atcagccggg acaacagcca gagcatcctg 300

tacctgcaga tgaacaccct gcgggccgag gactccgcca cctactactg cgccagagac 360

atccccggct tcgcctactg gggccagggc accctggtga ccgtgtccgc cgccagcacc 420

aagggcccaa gcgtgttccc gctagccccc agcagcaaga gcaccagcgg cggcacagcc 480

gccctgggct gcctggtgaa ggactacttc cccgagcccg tgaccgtgtc ctggaacagc 540

ggagccctga cctccggcgt gcacaccttc cccgccgtgc tgcagagcag cggcctgtac 600

agcctgagca gcgtggtgac cgtgcccagc agcagcctgg gcacccagac ctacatctgt 660

aacgtgaacc acaagcccag caacaccaag gtggacaaga gagtggagcc caagagctgt 720

gacaagaccc acacctgccc cccctgccca gcccccgagc tgctgggcgg acccagcgtg 780

ttcctgttcc cccccaagcc caaggacacc ctgatgatca gcagaacccc cgaggtgacc 840

tgtgtggtgg tggacgtgtc ccacgaggac ccagaggtga agttcaactg gtacgtggac 900

ggcgtggagg tgcacaacgc caagaccaag cccagagagg agcagtacaa cagcacctac 960

agggtggtgt ccgtgctgac cgtgctgcac caggactggc tgaacggcaa ggagtacaag 1020

tgtaaggtgt ccaacaaggc cctgccagcc ccaatcgaaa agaccatcag caaggccaag 1080

ggccagccaa gagagcccca ggtgtacacc ctgccaccca gcagggagga gatgaccaag 1140

aaccaggtgt ccctgacctg tctggtgaag ggcttctacc caagcgacat cgccgtggag 1200

tgggagagca acggccagcc cgagaacaac tacaagacca ccccccccagt gctggacagc 1260

gacggcagct tcttcctgta cagcaagctg accgtggaca agagcagatg gcagcagggc 1320

aacgtgttca gctgctccgt gatgcacgag gccctgcaca accactacac ccagaagagc 1380

ctgagcctgt ccccaggcaa gtga 1404

<210> 70
<211> 720
<212> DNA
<213> mus musculus

<400> 70
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gacattgtga tgtcacagtc tccatcctcc ctggctgtgt cagcaggaga gaaggtcact 120

atgagctgca aatccagtca gagtctgttc aacagtcgaa cccgaaagaa ctacttggct 180

tggtaccagc agaagccagg gcagtctcct aaactgctga tctactgggc atccgctagg 240

gattctgggg tccctgctcg cttcacaggc agtggatctg agacatattt cactctcacc 300

atcagccgtg tgcaggctga agacctggca gtttattact gcatgcaatc ttttaatctt 360

cggacgttcg gtggaggcac caagctggaa atcaaacgta cggtggccgc tcccagcgtg 420

ttcatcttcc ccccaagcga cgagcagctg aagagcggca ccgccagcgt ggtgtgtctg 480

ctgaacaact tctaccccag ggaggccaag gtgcagtgga aggtggacaa cgccctgcag 540

agcggcaaca gccaggagag cgtcaccgag caggacagca aggactccac ctacagcctg 600

agcagcaccc tgaccctgag caaggccgac tacgagaagc acaaggtgta cgcctgtgag 660

gtgacccacc agggcctgtc agccccgtg accaagagct tcaacagggg cgagtgctga 720

<210> 71
<211> 1410
<212> DNA
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