



HU000025083T2

(19) **HU****MAGYARORSZÁG**
Szellemi Tulajdon Nemzeti Hivatala(11) Lajstromszám: **E 025 083**(13) **T2****EURÓPAI SZABADALOM**
SZÖVEGÉNEK FORDÍTÁSA(21) Magyar ügyszám: **E 10 721376**(22) A bejelentés napja: **2010. 04. 29.**(51) Int. Cl.: **C07K 16/28** (2006.01)**A61K 393/95** (2006.01)**A61P 31/18** (2006.01)

(96) Az európai bejelentés bejelentési száma:

EP 20100721376

(97) Az európai bejelentés közzétételi adatai:

EP 2424897 A1 **2010. 11. 04.**

(86) A nemzetközi (PCT) bejelentési szám:

PCT/EP 10/055863

(97) Az európai szabadalom megadásának meghirdetési adatai:

EP 2424897 B1 **2015. 01. 07.**

(87) A nemzetközi közzétételi szám:

WO 10125162

(30) Elsőbbségi adatok:

09159076 **2009. 04. 29.** **EP****173680 P** **2009. 04. 29.** **US**

(73) Jogosult(ak):

Pierre Fabre Medicament, 92100**Boulogne-Billancourt (FR)**

(72) Feltaláló(k):

KLINGUER-HAMOUR, Christine, F-74570 Groisy (FR)

(74) Képviselő:

dr. Kompagne Hajnalka, DANUBIA Szabadalmi és Jogi Iroda Kft., Budapest

(54)

Anti-CXCR4 ellenanyagok HIV kezelésére

Az európai szabadalom ellen, megadásának az Európai Szabadalmi Közlönyben való meghirdetésétől számított kilenc hónapon belül, felszólalást lehet benyújtani az Európai Szabadalmi Hivatalnál. (Európai Szabadalmi Egyezmény 99. cikk(1))

A fordítást a szabadalmas az 1995. évi XXXIII. törvény 84/H. §-a szerint nyújtotta be. A fordítás tartalmi helyességét a Szellemi Tulajdon Nemzeti Hivatala nem vizsgálta.



(11) **EP 2 424 897 B1**

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention
of the grant of the patent:
07.01.2015 Bulletin 2015/02

(51) Int Cl.:
C07K 16/28 ^(2006.01) **A61K 39/395** ^(2006.01)
A61P 31/18 ^(2006.01)

(21) Application number: **10721376.1**

(86) International application number:
PCT/EP2010/055863

(22) Date of filing: **29.04.2010**

(87) International publication number:
WO 2010/125162 (04.11.2010 Gazette 2010/44)

(54) **ANTI CXCR4 ANTIBODIES FOR THE TREATMENT OF HIV**

ANTIKÖRPER GEGEN CXCR4 ZUR BEHANDLUNG VON HIV

ANTICORPS ANTI CXCR4 POUR LE TRAITEMENT DU VIH

(84) Designated Contracting States:
**AT BE BG CH CY CZ DE DK EE ES FI FR GB GR
HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL
PT RO SE SI SK SM TR**
Designated Extension States:
AL BA ME RS

(30) Priority: **29.04.2009 EP 09159076**
29.04.2009 US 173680 P

(43) Date of publication of application:
07.03.2012 Bulletin 2012/10

(73) Proprietor: **Pierre Fabre Médicament**
92100 Boulogne-Billancourt (FR)

(72) Inventor: **KLINGUER-HAMOUR, Christine**
F-74570 Groisy (FR)

(74) Representative: **Regimbeau**
20, rue de Chazelles
75847 Paris Cedex 17 (FR)

(56) References cited:

- **TANAKA R ET AL:** "Unique monoclonal antibody recognizing the third extracellular loop of CXCR4 induces lymphocyte agglutination and enhances human immunodeficiency virus type 1-mediated syncytium formation and productive infection." JOURNAL OF VIROLOGY DEC 2001, vol. 75, no. 23, December 2001 (2001-12), pages 11534-11543, XP002548956 ISSN: 0022-538X
- **STRIZKI J M ET AL:** "A monoclonal antibody (12G5) directed against CXCR-4 inhibits infection with the dual-tropic human immunodeficiency virus type 1 isolate HIV-1(89.6) but not the T-tropic isolate HIV-1(HxB)." JOURNAL OF VIROLOGY JUL 1997, vol. 71, no. 7, July 1997 (1997-07), pages 5678-5683, XP002548957 ISSN: 0022-538X
- **REY-CUILLE M A ET AL:** "Conserved CXCR4 usage and enhanced replicative capacity of HIV-2/287, an isolate highly pathogenic in Macaca nemestrina." AIDS (LONDON, ENGLAND) 7 DEC 2001, vol. 15, no. 18, 7 December 2001 (2001-12-07), pages 2349-2357, XP002548958 ISSN: 0269-9370
- **MONDOR I ET AL:** "Interaction among HIV gp120, CD4, and CXCR4: dependence on CD4 expression level, gp120 viral origin, conservation of the gp120 COOH- and nh2- termini and V1/V2 and V3 loops, and sensitivity to neutralizing antibodies" VIROLOGY, ACADEMIC PRESS, ORLANDO, US, vol. 248, no. 2, 1 September 1998 (1998-09-01), pages 394-405, XP002086992 ISSN: 0042-6822

Note: Within nine months of the publication of the mention of the grant of the European patent in the European Patent Bulletin, any person may give notice to the European Patent Office of opposition to that patent, in accordance with the Implementing Regulations. Notice of opposition shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

Description

[0001] 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, and functional fragments thereof, able to bind specifically to the CXCR4 and having strong activity against human immunodeficiency virus (HIV) infection. The invention also comprises the use of such antibodies, and functional fragments thereof as a medicament for the preventive and/or therapeutic treatment of HIV infection.

[0002] 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 NH₂-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.

[0003] Several members of the chemokine receptor family function as co-receptors with the primary receptor CD4 to allow entry of various strains of HIV type 1 into the cells, the major co-receptors being CCR5 and CXCR4. T-cell tropic X4 HIV-1 use CD4 and CXCR4 for the entry into the cells, whereas macrophage-tropic R5 HIV-1 use CD4 and CCR5. Dual-tropic strains can use both CXCR4 and CCR5 as co-receptors. CCR3, CCR2, CCR8, CXCR6, CXCR7, CX3CR1 among other chemokine receptors can function as co-receptors by more restricted subset of HIV strains.

[0004] SDF-1, the natural ligand of CXCR4 as well as CCL3, CCL4, CCL4-L1, and CCL5 ligands for CCR5 are able to inhibit cell fusion and infection by various strains of HIV-1. These findings have encouraged the development of anti-HIV therapeutics targeting chemokine receptors leading to the approval of maraviroc (CELESTRI®), a small molecule antagonist of CCR5 in combination with other anti-HIV-1 agents in patients infected by CCR5-tropic HIV-1. Nevertheless, maraviroc is neither used in patients infected by dual-tropic HIV-1 nor in patients infected by CXCR4-tropic HIV-1 (VIDAL 2009). So there is a clear medical need to extend this type of therapy in both X4-tropic and dual-tropic HIV infected patients by identifying CXCR4 antagonists able to inhibit X4-tropic HIV replication.

[0005] 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).

[0006] This receptor is expressed by different kind of normal tissues, naive, 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.

[0007] 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.

[0008] As previously mentioned, CXCR4 receptor is the principal co-receptor for T-cell-tropic HIV-1 isolates (X4 viruses). Interfering with this receptor should inhibit X4 viruses replication in a very efficient way.

[0009] One of the inventive aspect of the present invention is to generate mouse monoclonal antibodies (Mabs) inhibiting HIV replication. The invention encompasses a CXCR4 Mab 515H7 (or fragments thereof) able to bind to CXCR4 homodimers, and having strong activities against HIV infection. The invention also encompasses a CXCR4 Mab 301aE5 (or fragments thereof) able to bind to CXCR4 homodimers, and having strong activities against HIV infection.

[0010] Surprisingly, inventors have managed to generate monoclonal antibodies capable of binding to CXCR4 but also capable of inducing conformational changes of the CXCR4 homodimers and able to inhibit primary isolate X4-HIV-1 replication in PBMC. More particularly, the antibodies of the invention can also be able to inhibit primary isolate X4/R5-HIV-1 replication in PBMC.

[0011] Preferably, CXCR4 compound 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. 27: MEGISYTSNDNYTEEMGSGDYDSMKEPCFREENANFNKIFLPTIYSIIFLTGIVGN GLVILVMGYQKKLRSMTDKYRLHLSVADLLFVITLPFWAVDAVANWYFGNFLCKAVHVIYTVNLYSSVLILAFISLDRYLAIVHATNSQRPRKLLAEKVYVGVWIPALLLTIPDFIFANVSEADDRYICDRFYPNDLWVVVFQFHIMVGLILPGIVILSCYCIISKLSHSGKHQKRKALKTTVILILAFFACWLPYYIGISIDSFILLEIIKQGCEFE NTVHKWISITEALAFFHCCLNPILYAFLGAKFKTSAQHALTSVSRGSSLKILSKG KRGGHSSVSTESSESSSFHSS;
- 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. 28: MSIPLPLLQIYTSNDNYTEEMGSGDYDSMKEPCFREENANFNKIFLPTIYSIIFLTGI VGNGLVILVMGYQKKLRSMTDKYRLHLSVADLLFVITLPFWAVDAVANWYFG

NFLCKAVHVIYTVNLYSSVLILAFISLDRYLAIVHATNSQRPRKLLAEKVYVVG
VWIPALLLTIPDFIFANVSEADDRYICDRFYPNDLWVVVFQFQHIMVGLILPGIVI
LSCYCIISKLSHSGHQKRKALKTTVILILAFFACWLPYYIGISIDSFILLEIKQGC
EFENTVHKWISITEALAFFHCCLNPILYAFLGAKFKTSAQHALTSVSRGSSLKILS KGKRGGHSSVSTESESSS-
FHSS;

- 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. 27 or 28; 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.

[0012] CXCR2 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. 29: MEDFNMESDSFEDFWKGEDLSNYSYSSTLPPFLDDAAPCEPESLEINKYFV-VIIY ALVFLLSLLGNSLVMLVILYSRVGRSVTDVYLLNLALADLLFALTLPWAASKV NGWIFGTFLCKVVSLLKEVNFYSGILLACISVDYLAIVHATRTLTKRYLVKF ICLSIWGLSLLLALPVLLFRRTVYSSNVSPACYEDMGNNNTANWRMLLRILPQSF GFIVPLLIIMLCYGFTRLTLFKAHMGQKHRAMRVIFAVVLIFLLCWLPYNLVLL ADTLMRTQVIQETCERRNHIDRALDATEILGILHSCLNPLIYAFIGQKFRHGLLKI LAIHGLISKDSLKPDSRPS-FVGSSSGHTSTTL;
- 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. 29; and
- a fragment thereof capable of being specifically recognizing by IL-8 and having preferably at least 100, 150 and 200 amino acid length.

[0013] The present invention also relates to a method for selecting a compound having an anti-HIV activity or which can be used for the preparation of a composition for the treatment of HIV infection, characterized in that said method comprises the step of:

In a first aspect, is the present disclosure relates to a process for the generation and the selection of antibodies according to the invention.

[0014] More particularly, a process for the selection of an anti CXCR4 antibody, or one of its functional fragments, capable to inhibit HIV replication is herein disclosed, said process comprising the following steps:

- i) screening the generated antibodies and selecting antibodies capable to bind specifically to CXCR4;
- ii) testing the selected antibodies of step i) and selecting antibodies capable to bind peripheral blood mononuclear cells (PBMC),
- iii) testing the selected antibodies of step ii) and selecting antibodies capable to bind to CXCR4 homodimer, and then
- iv) testing the selected antibodies of step iii) and selecting antibodies capable to inhibit primary isolates X4-tropic HIV-1 replication in PBMC.

[0015] In another embodiment, the present disclosure concerns a process for the selection of an anti CXCR4 antibody, or one of its functional fragments, capable to inhibit HIV replication comprising the following steps:

- i) screening the generated antibodies and selecting antibodies capable to bind specifically to CXCR4;
- ii) testing the selected antibodies of step i) and selecting antibodies capable to bind peripheral blood mononuclear cells (PBMC),
- iii) testing the selected antibodies of step ii) and selecting antibodies capable to bind to CXCR4 homodimer, and then
- iv) testing the selected antibodies of step iii) and selecting antibodies capable to inhibit primary isolates X4-tropic HIV-1 replication in PBMC and/or capable to inhibit primary isolates X4/R5-tropic HIV-1 replication in PBMC.

[0016] The generation of the antibodies 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.

[0017] The screening steps i) and ii) 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, western blot analysis using CXCR4 expressing cell membrane extracts or purified CXCR4, FACS analysis and functional screens. A preferred process consists in a screen by FACS analysis on CXCR4 transfectant (step 1) and on at least PBMC (step 2) to be sure that the produced antibodies will be able to also recognize the native CXCR4 receptor conformation at the target cell surface. This process will be described more precisely in the following examples.

[0018] The screening step iii) can be realized by any method or process known by the man skilled in the art. As non limiting but preferred example, can be mentioned western blotting and/or immuno-precipitation techniques using antibodies of interest on membrane extract from CXCR4 transfected cells or PBMC.

[0019] The screening step iv) can be realized by any method or process known by the man skilled in the art. As non limitative but preferred example, can be mentioned a process consisting in screening antibodies for their ability to inhibit X4 primary HIV-1 and/or X4/R5 primary HIV-1 isolates replication in PBMC by using a protocol described by Holl et al. (J. Immunol. 2004, 173, 6274-83).

[0020] In a preferred embodiment of the step iii) of selection of the process disclosed herein, said step iii) consists in evaluating antibodies by BRET analysis on cells expressing CXCR4-RLuc/CXCR4-YFP and selecting antibodies capable to inhibit at least 40 %, preferably 45 %, 50 %, 55 % and most preferably 60 % of the BRET signal.

[0021] The BRET technology is a technology known as being representative of the protein dimerization [Angers et al., PNAS, 2000, 97:3684-89].

[0022] The BRET technology, used in the step 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 bio luminescent 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.

[0023] 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).

[0024] 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.

[0025] In a particular aspect of the said process, step iii) 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.

[0026] In a second aspect, the present disclosure relates to isolated antibodies, or one of their functional fragments thereof, being obtained by said process. Said antibodies or one of their said fragments or derivatives, are capable of binding specifically to the human CXCR4, said antibodies being also capable to induce CXCR4 homodimers conformational changes.

[0027] It is known from literature that CXCR4 Mabs like, for example clone A120, are able to inhibit HIV-1 laboratory strain (X4HIV-1_{NL4-3}) entry into PBMC (Tanaka R. et al, J. Virol. 2001, 75, 11534-11543). Furthermore, it is also known that CXCR4 Mabs are able to inhibit HIV-1 X4 primary isolates into cell lines expressing CXCR4. On the contrary, it has never been disclosed antibody able to inhibit such virus in their natural environment, i.e. not only on laboratory viruses or cell lines. Nevertheless, it is a novel and not obvious aspect of the invention that CXCR4 Mabs are able to inhibit HIV-1 X4 primary isolates into PBMC.

[0028] The expressions "functional fragments" will be defined in details later in the present specification.

[0029] More particularly, it is claimed isolated antibodies, or one of their functional fragments or derivatives, said antibodies being characterized in that they comprise at least one complementary determining region CDR chosen from CDRs comprising the amino acid sequence SEQ ID Nos. 1 to 6 and 30 to 33 as defined by the IMGT numbering system.

[0030] According to a first aspect, the disclosure relates to an isolated antibody, or a functional fragment or derivative of same, comprising at least one CDR chosen among the CDRs of sequences SEQ ID Nos. 1 to 6, as defined according to the IMGT numbering system, 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. 1 to 6.

[0031] According to a second aspect, the disclosure relates to an isolated antibody, or a functional fragment or derivative

of same, comprising at least one CDR chosen among the CDRs of sequences SEQ ID Nos. 1, 2 and 30 to 33, as defined according to the IMGT numbering system, 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. 1, 2 and 30 to 33.

[0032] 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.

[0033] 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.

[0034] More preferably, the invention comprises the antibodies, and the functional fragments thereof, according to the present invention, notably chimeric or humanized, obtained by genetic recombination or chemical synthesis.

[0035] According to a preferred embodiment, the antibody according to the invention, or its functional fragments, is characterized in that it consists of a monoclonal antibody.

[0036] "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.

[0037] More particularly, the antibody, or its derived compounds or functional fragments, is characterized in that it comprises a light chain comprising at least one CDR chosen from CDR-L1, CDR-L2 and CDR-L3, wherein:

- CDR-L1 comprises the amino acid sequence SEQ ID No. 1,
- CDR-L2 comprises the amino acid sequence SEQ ID No. 2,
- CDR-L3 comprises the amino acid sequence SEQ ID No. 3.

[0038] According to another embodiment, the antibodies, 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. 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.

[0039] The antibody, or one of its functional fragments or derivatives, is also characterized in that it comprises a light chain comprising CDR-L1, CDR-L2 and CDR-L3, wherein CDR-L1 comprises the amino acid sequence SEQ ID No. 1, CDR-L2 comprises the amino acid sequence SEQ ID No. 2 and CDR-L3 comprises the amino acid sequence SEQ ID No. 3.

[0040] In another embodiment, the antibody, or one of its functional fragments or derivatives, is characterized in that it comprises a light chain of sequence comprising the amino acid sequence SEQ ID No. 7, or at least one sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID No. 7.

[0041] According to a second preferred embodiment, the antibody, or its derived compounds or functional fragments, is characterized in that it comprises a light chain comprising at least one CDR chosen from CDR-L1, CDR-L2 and CDR-L3, wherein:

- CDR-L1 comprises the amino acid sequence SEQ ID No. 1,
- CDR-L2 comprises the amino acid sequence SEQ ID No. 2,
- CDR-L3 comprises the amino acid sequence SEQ ID No. 30.

[0042] According to another embodiment, the antibodies, 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. 1, 2 or 30, 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 30.

[0043] The antibody, or one of its functional fragments or derivatives, is also characterized in that it comprises a light chain comprising CDR-L1, CDR-L2 and CDR-L3, wherein CDR-L1 comprises the amino acid sequence SEQ ID No. 1, CDR-L2 comprises the amino acid sequence SEQ ID No. 2 and CDR-L3 comprises the amino acid sequence SEQ ID No. 30.

[0044] In another embodiment, the antibody, or one of its functional fragments or derivatives, is characterized in that it comprises a light chain of sequence comprising the amino acid sequence SEQ ID No. 34, or at least one sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID No. 34.

[0045] More particularly, the antibodies, or one of their derived compounds or functional fragments, are characterized in that they comprise a heavy chain comprising at least one CDR chosen from CDR-H1, CDR-H2 and CDR-H3, wherein:

- CDR-H1 comprises the amino acid sequence SEQ ID No. 4,
- CDR-H2 comprises the amino acid sequence SEQ ID No. 5,
- CDR-H3 comprises the amino acid sequence SEQ ID No. 6.

[0046] According to another embodiment, the antibodies, 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.

[0047] 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 CDR-H1, CDR-H2 and CDR-H3, wherein CDR-H1 comprises the amino acid sequence SEQ ID No. 4, CDR-H2 comprises the amino acid sequence SEQ ID No. 5 and the CDR-H3 comprises the amino acid sequence SEQ ID No. 6.

[0048] In another embodiment, the antibody, or one of its functional fragments or derivatives, is characterized in that it comprises a heavy chain of sequence comprising the amino acid sequence SEQ ID No. 8, or at least one sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID No. 8.

[0049] More particularly, the said antibodies, or one of their derived compounds or functional fragments, are characterized in that they comprise a heavy chain comprising at least one CDR chosen from CDR-H1, CDR-H2 and CDR-H3, wherein:

- CDR-H1 comprises the amino acid sequence SEQ ID No. 31,
- CDR-H2 comprises the amino acid sequence SEQ ID No. 32,
- CDR-H3 comprises the amino acid sequence SEQ ID No. 33.

[0050] 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 heavy chain comprising at least one of the three CDRs of the sequences SEQ ID Nos. 31, 32 or 33, or at least one sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID Nos. 31, 32 or 33.

[0051] 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 CDR-H1, CDR-H2 and CDR-H3, wherein CDR-H1 comprises the amino acid sequence SEQ ID No. 31, CDR-H2 comprises the amino acid sequence SEQ ID No. 32 and the CDR-H3 comprises the amino acid sequence SEQ ID No. 33.

[0052] In another embodiment, the said antibody, or one of its functional fragments or derivatives, is characterized in that it comprises a heavy chain of sequence comprising the amino acid sequence SEQ ID No. 35, or at least one sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID No. 35.

[0053] The antibody of the invention, or one of its functional fragments, is characterized in that it comprises a light chain comprising CDR-L1, CDR-L2 and CDR-L3 comprising respectively the amino acid sequence SEQ ID Nos. 1, 2 and 3; and a heavy chain comprising CDR-H1, CDR-H2 and CDR-H3 comprising respectively the amino acid sequence SEQ ID Nos. 4, 5 and 6.

[0054] The antibody of the invention, or one of its functional fragments, can also be characterized in that it comprises a light chain comprising the amino acid sequence SEQ ID No. 7 and a heavy chain comprising the amino acid sequence SEQ ID No. 8.

[0055] The antibody of the invention, or one of its functional fragments, is characterized in that it comprises a light chain comprising CDR-L1, CDR-L2 and CDR-L3 comprising respectively the amino acid sequence SEQ ID Nos. 1, 2 and 30; and a heavy chain comprising CDR-H1, CDR-H2 and CDR-H3 comprising respectively the amino acid sequence SEQ ID Nos. 31, 32 and 33.

[0056] The antibody of the invention, or one of its functional fragments, can also be characterized in that it comprises a light chain comprising the amino acid sequence SEQ ID No. 34 and a heavy chain comprising the amino acid sequence SEQ ID No. 35.

[0057] In the present description, the terms "polypeptides", "polypeptide sequences", "peptides" and "proteins attached to antibody compounds or to their sequences" are interchangeable.

[0058] 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.

[0059] 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.

[0060] 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.

[0061] 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)].

[0062] 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.

[0063] 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 system and by Kabat numbering system.

[0064] 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.

[0065] More particularly, concerning the antibody referred as 515H7, CDR-L1 consists of SEQ ID No. 1 in the IMGT numbering systems and of SEQ ID No. 9 in the Kabat numbering system. Concerning the CDR-L2, it consists of SEQ ID No. 2 in the IMGT numbering systems and of SEQ ID No. 10 in the Kabat numbering system. The CDR-L3 consists of SEQ ID No. 3 for each of the two numbering systems. For the heavy chain, the CDR-H1 consists of the SEQ ID No. 4 in the IMGT numbering system and of SEQ ID No. 11 in the kabat numbering system. The CDR-H2 consists of SEQ ID No. 5 in the IMGT numbering systems and of SEQ ID No. 12 in the kabat numbering system. At last, the CDR-H3 consists in the SEQ ID No. 6 in the IMGT numbering systems whereas it consists of SEQ ID No. 13 in the kabat numbering system.

[0066] Then, concerning the antibody referred as 301aE5, CDR-L1 consists of SEQ ID No. 1 in the IMGT numbering systems and of SEQ ID No. 9 in the Kabat numbering system. Concerning the CDR-L2, it consists of SEQ ID No. 2 in the IMGT numbering systems and of SEQ ID No. 36 in the Kabat numbering system. The CDR-L3 consists of SEQ ID No. 30 in the IMGT numbering systems and of SEQ ID No. 37 in the Kabat numbering system. For the heavy chain, the CDR-H1 consists of the SEQ ID No. 31 in the IMGT numbering system and of SEQ ID No. 38 in the Kabat numbering system. The CDR-H2 consists of SEQ ID No. 32 in the IMGT numbering systems and of SEQ ID No. 39 in the Kabat numbering system. At last, the CDR-H3 consists in the SEQ ID No. 33 in the IMGT numbering systems whereas it consists of SEQ ID No. 40 in the Kabat numbering system.

[0067] As used herein, 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 Needleman 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).

[0068] 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.

[0069] 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.

[0070] 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.

[0071] 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

(continued)

Original residue	Substitution(s)
Val (V)	Leu, Ala

5

[0072] In a specific embodiment, the present invention relates to murine antibodies, or derived compounds or functional fragments of same.

[0073] The present disclosure also relates to any compound derived from antibodies as described in the invention.

10

[0074] More particularly, it is herein disclosed an antibody, or its derived compounds or functional fragments, 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.

[0075] One or more sequences among the CDR sequences described herein 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.

15

[0076] 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):

20

- 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.

25

[0077] 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".

30

[0078] Scaffolds derived from toxins such as, for example, toxins from scorpions, insects, plants, mollusks, etc., and the protein inhibitors of neuronal NO synthase (PIN) should also be mentioned.

[0079] An example, in no way limiting, of such hybrid constructions, is the insertion of the CDR-H1 (heavy chain) of an anti-CD4 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 neocarainostatin (Nicaise et al., Protein Science, 2004, 13(7):1882-1891) can also be mentioned.

35

[0080] Lastly, as described above, such peptide scaffolds can comprise at least one of the 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).

40

[0081] A method for selecting a compound derived from an antibody according to the invention is provided, said derived compound being capable of inhibiting *in vitro* and/or *in vivo* HIV cell entry and said derived compound comprising a peptide scaffold on which is grafted at least one antibody CDR, said method comprising the following steps:

45

- 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 HIV type 1 and PBMC; and
- b) selection of said compound if said compound is capable of inhibiting the HIV-1 replication,

50

and characterized in that said at least one grafted CDR is selected among the following CDRs of sequence SEQ ID No. 1 to 6 and 30 to 33 or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID Nos. 1 to 6 and 30 to 33.

55

[0082] 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.

[0083] 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.

[0084] 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.

[0085] The present disclosure 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.

[0086] According to a preferred embodiment, the antibody, 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 inhibitors of neuronal NO synthase (PIN).

[0087] Another aspect of the invention relates to the functional fragments of the antibody described above.

[0088] 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.

[0089] Such functional fragments of the antibody according to the invention consist, for example, of the fragments Fv, scFv (sc=single 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.

[0090] 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.

[0091] 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.

[0092] 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.

[0093] For more clarity, table 2 below summarizes the various amino acid sequences corresponding to the antibodies of the invention.

Table 2 (wherein Mu. = murine)

Antibody	CDR numbering	Heavy chain	Light chain	SEQ ID NO.
515H7	IMGT		CDR-L1	1
			CDR-L2	2
			CDR-L3	3
		CDR-H1		4
		CDR-H2		5
		CDR-H3		6
	Kabat		CDR-L1	9
			CDR-L2	10
			CDR-L3	3
		CDR-H1		11
		CDR-H2		12
		CDR-H3		13
			Mu. variable domain	7
		Mu. variable domain		8
301aE5	IMGT		CDR-L1	1
			CDR-L2	2
			CDR-L3	30
		CDR-H1		31
		CDR-H2		32
		CDR-H3		33
	Kabat		CDR-L1	9
			CDR-L2	36
			CDR-L3	37
		CDR-H1		38
		CDR-H2		39
		CDR-H3		40
			Mu. variable domain	34
		Mu. variable domain		35

[0094] A particular important additional aspect of the antibodies object of the present invention is that they do not exhibit effector functions, such as antibody-dependent cellular cytotoxicity (ADCC) and/or complement-dependant cytotoxicity (CDC).

[0095] More particularly, as an example, the antibodies of the invention, or one of their functional fragments thereof, have no affinity for the Fc γ R (I, II or III) or for the C1q, or for both of them.

[0096] Structurally, this means for the man skilled in the art that the antibodies of the invention, or one of their functional fragments thereof, are devoid of Fc portion or their Fc portion does not present a correct glycosylation able to confer effector functions.

[0097] Consequence of this is that the antibodies of the invention are preferably selected from IgG4 or IgG2 isotypes, most preferably IgG4.

[0098] Similarly, preferred fragments are fragments devoid of ADCC such as Fv, scFv (sc for single chain), Fab, F(ab')₂, Fab', scFv-Fc fragments or diabodies, or any fragment of which the half-life time would have been increased by chemical modification, such as the addition of poly(alkylene) glycol such as poly(ethylene) glycol ("PEGylation")

(pegylated fragments called Fv-PEG, scFv-PEG, Fab-PEG, F(ab')₂-PEG or Fab'-PEG) ("PEG" for Poly(Ethylene) Glycol), or by incorporation in a liposome.

[0099] More particularly, a preferred functional fragment of the invention derived from the antibody 515H7 is a scFv, hereinafter referred as 515H7 scFv-Ck fragment, comprises the amino acid sequence SEQ ID No. 54

[0100] The nucleotide sequence corresponding to said scFv comprises the sequence SEQ ID No. 55.

[0101] Another specific aspect of the present invention relates to chimeric antibodies, or their derived compounds or functional fragments, characterized in that said antibodies also comprise light-chain and heavy-chain constant regions derived from an antibody of a species heterologous with the mouse, notably man.

[0102] Yet another specific aspect of the present invention relates to humanized antibodies, or their 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-2 or preferably gamma-4 region.

[0103] The antibody of the invention also comprises chimeric or humanized antibodies.

[0104] 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.

[0105] 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 Verhoeven et al. (BioEssays, 8:74, 1988) for methods for preparing chimeric antibodies.

[0106] The table 3 herein-under summarizes the amino acids sequences of the various heavy and light chains of the chimeric antibody 515H7 (referred as c515H7 or C515H7) according to the invention.

Table 3 (wherein c = chimeric)

Antibody c515H7	Heavy chain	Light chain	SEQ ID NO.
Complete Sequences (without signal peptide)	cVH (G4wt)	-	56
	cVH (G4PRO)	-	57
	cVH (G2 wt)	-	58
	-	cVL-Ck	59

[0107] The nucleotide sequence corresponding to said Antibody c515H7 heavy chains SEQ ID Nos. 56 to 58 and light chain SEQ ID No. 59 correspond respectively to the sequence SEQ ID Nos. 60 to 63 (heavy chains) and SEQ ID No. 64 (light chain).

[0108] In a preferred embodiment, the heavy chain sequences are deleted from their C-terminal lysine residue (as found in the original pConPlus vector series from Lonza: pConPlusγ4ΔK, pConPlusγ4PROΔK & pConPlusγ2ΔK).

[0109] Moreover, the G4PRO heavy chain corresponds to a human IgG4 isotype carrying a mutation in the Hinge region to avoid formation of half-antibodies. This mutation is found in the parental pConPlusγ4PROΔK from Lonza [Angal S, King DJ, Bodmer MW, Turner A, Lawson AD, Roberts G, Pedley B, Adair JR. A single amino acid substitution abolishes the heterogeneity of chimeric mouse/human (IgG4) antibody. Mol Immunol. (1993);30(1):105-108].

[0110] More particularly, the chimeric antibody heavy chain comprises CDRs homologous to corresponding CDRs of an antibody derived from a different mammalian species, wherein said CDRs, according to IMGT, consist of CDR-H1, CDR-H2 and CDR-H3 comprising respectively the sequences SEQ ID Nos. 4, 5 and 6.

[0111] More particularly, the chimeric antibody light chain comprises CDRs homologous to corresponding CDRs of an antibody derived from a different mammalian species, wherein said CDRs, according to IMGT, consist of CDR-L1, CDR-L2 and CDR-L3 comprising respectively the sequences SEQ ID Nos. 1, 2 and 3.

[0112] More particularly, the invention relates to a chimeric antibody, or a functional fragment of same, characterized in that it comprises heavy and light chains each having CDRs homologous to corresponding CDRs of an antibody derived from a different mammalian species, wherein said CDRs, according to IMGT, consist of CDR-H1, CDR-H2 and CDR-H3 of the heavy chain comprising respectively the sequences SEQ ID Nos. 4, 5 and 6, and CDR-L1, CDR-L2 and CDR-L3 of the light chain comprising respectively the sequences SEQ ID Nos. 1, 2 and 3.

[0113] In another embodiment, the invention relates to a chimeric antibody, or a functional fragment of same, comprising a heavy chain variable region of sequence consisting of SEQ ID No. 8, and a light chain variable region of sequence SEQ ID No. 7.

[0114] In still another embodiment, the invention relates to a chimeric antibody, or a functional fragment of same, comprising a heavy chain of sequence selected from the group consisting of SEQ ID Nos. 56, 57 or 58, and a light chain of sequence SEQ ID No. 59.

[0115] In a preferred embodiment, the chimeric antibody c515H7 VH(G4wt) / VL-Ck, or a functional fragment of same, according to the invention comprises a heavy chain variable region of sequence SEQ ID No. 56, and a light chain variable region of sequence SEQ ID No. 59.

[0116] In a preferred embodiment, the chimeric antibody c515H7 VH(G4PRO) / VL-Ck, or a functional fragment of same, according to the invention comprises a heavy chain variable region of sequence SEQ ID No. 57, and a light chain variable region of sequence SEQ ID No. 59.

[0117] In a preferred embodiment, the chimeric antibody c515H7 VH(G2wt) / VL-Ck, or a functional fragment of same, according to the invention comprises a heavy chain variable region of sequence SEQ ID No. 58, and a light chain variable region of sequence SEQ ID No. 59.

[0118] "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).

[0119] 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.

[0120] In addition, the invention also relates to humanized antibodies arising from the murine antibody described above.

[0121] 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-2 or preferably gamma-4 region.

[0122] More particularly, the invention relates to a humanized antibody heavy chain characterized in that it comprises i) a framework region homologous to corresponding framework region of a human antibody heavy chain, and ii) CDRs homologous to corresponding CDRs of an antibody derived from a different mammalian species, wherein said CDRs, according to IMGT, consist of CDR-H1, CDR-H2 and CDR-H3 comprising respectively the sequences SEQ ID Nos. 4, 5 and 6.

[0123] In another embodiment, the invention relates to a humanized antibody heavy chain comprising a variable region of sequence consisting of SEQ ID No. 64.

[0124] In still another embodiment, the invention relates to a humanized antibody heavy chain comprising the complete sequence selected from the group consisting of SEQ ID Nos. 67, 68 and 69.

[0125] More particularly, the invention relates to a humanized antibody light chain characterized in that it comprises i) a framework region homologous to corresponding framework region of a human antibody light chain, and ii) CDRs homologous to corresponding CDRs of an antibody derived from a different mammalian species, wherein said CDRs, according to IMGT, consist of CDR-L1, CDR-L2 and CDR-L3 comprising respectively the sequences SEQ ID Nos. 1, 2 and 3.

[0126] In another embodiment, the invention relates to a humanized antibody light chain comprising a variable region of sequence selected from the group consisting of SEQ ID Nos. 65, 66, 82 or 83.

[0127] In still another embodiment, the invention relates to a humanized antibody light chain comprising the complete sequence selected from the group consisting of SEQ ID Nos. 70, 71, 84 or 85.

[0128] More particularly, the invention relates to a humanized antibody, or a functional fragment of same, characterized in that it comprises heavy and light chains each having i) framework regions homologous to corresponding framework regions of a human antibody, and ii) CDRs homologous to corresponding CDRs of an antibody derived from a different mammalian species, wherein said CDRs, according to IMGT, consist of CDR-H1, CDR-H2 and CDR-H3 of the heavy chain comprising respectively the sequences SEQ ID Nos. 4, 5 and 6, and CDR-L1, CDR-L2 and CDR-L3 of the light chain comprising respectively the sequences SEQ ID Nos. 1, 2 and 3.

[0129] In another embodiment, the invention relates to a humanized antibody, or a functional fragment of same, comprising a heavy chain variable region of sequence consisting of SEQ ID No. 64, and a light chain variable region of sequence selected from the group consisting of SEQ ID Nos. 65, 66, 82 or 83.

[0130] In still another embodiment, the invention relates to a humanized antibody, or a functional fragment of same, comprising a heavy chain of sequence selected from the group consisting of SEQ ID Nos. 67, 68 or 69, and a light chain of sequence selected from the group consisting of SEQ ID Nos. 70, 71, 84 or 85.

[0131] In a preferred embodiment, the humanized antibody Hz515H7 VH1 D76N (G4wt) / VL2-Ck, or a functional

fragment of same, according to the invention comprises a heavy chain of sequence SEQ ID No. 67, and a light chain of sequence SEQ ID No. 70.

[0132] In another preferred embodiment, the humanized antibody Hz515H7 VH1 D76N (G4PRO) / VL2-Ck, or a functional fragment of same, according to the invention comprises a heavy chain of sequence SEQ ID No. 68, and a light chain of sequence SEQ ID No. 70.

[0133] In another preferred embodiment, the humanized antibody Hz515H7 VH1 D76N (G2wt) / VL2-Ck, or a functional fragment of same, according to the invention comprises a heavy chain of sequence SEQ ID No. 69, and a light chain of sequence SEQ ID No. 70.

[0134] In another preferred embodiment, the humanized antibody Hz515H7 VH1 D76N (G4wt) / VL2.1-Ck, or a functional fragment of same, according to the invention comprises a heavy chain of sequence SEQ ID No. 67, and a light chain of sequence SEQ ID No. 71.

[0135] In another preferred embodiment, the humanized antibody Hz515H7 VH1 D76N (G4PRO) / VL2.1-Ck, or a functional fragment of same, according to the invention comprises a heavy chain of sequence SEQ ID No. 68, and a light chain of sequence SEQ ID No. 71.

[0136] In another preferred embodiment, the humanized antibody Hz515H7 VH1 D76N (G2wt) / VL2.1-Ck, or a functional fragment of same, according to the invention comprises a heavy chain of sequence SEQ ID No. 69, and a light chain of sequence SEQ ID No. 71.

[0137] In another preferred embodiment, the humanized antibody Hz515H7 VH1 D76N (G4wt) / VL2.2-Ck, or a functional fragment of same, according to the invention comprises a heavy chain of sequence SEQ ID No. 67, and a light chain of sequence SEQ ID No. 84.

[0138] In another preferred embodiment, the humanized antibody Hz515H7 VH1 D76N (G4PRO) / VL2.2-Ck, or a functional fragment of same, according to the invention comprises a heavy chain of sequence SEQ ID No. 68, and a light chain of sequence SEQ ID No. 84.

[0139] In another preferred embodiment, the humanized antibody Hz515H7 VH1 D76N (G2wt) / VL2.2-Ck, or a functional fragment of same, according to the invention comprises a heavy chain of sequence SEQ ID No. 69, and a light chain of sequence SEQ ID No. 84.

[0140] In another preferred embodiment, the humanized antibody Hz515H7 VH1 D76N (G4wt) / VL2.3-Ck, or a functional fragment of same, according to the invention comprises a heavy chain of sequence SEQ ID No. 67, and a light chain of sequence SEQ ID No. 85.

[0141] In another preferred embodiment, the humanized antibody Hz515H7 VH1 D76N (G4PRO) / VL2.3-Ck, or a functional fragment of same, according to the invention comprises a heavy chain of sequence SEQ ID No. 68, and a light chain of sequence SEQ ID No. 85.

[0142] In another preferred embodiment, the humanized antibody Hz515H7 VH1 D76N (G2wt) / VL2.3-Ck, or a functional fragment of same, according to the invention comprises a heavy chain of sequence SEQ ID No. 69, and a light chain of sequence SEQ ID No. 85.

[0143] The table 4 herein-under summarizes the amino acids sequences of the various heavy and light chains variable domains and full length (or complete), respectively, of the humanized antibody 515H7 according to the invention.

Table 4 (wherin Hz = humanized)

Antibody Hz515H7	Heavy chain	Light chain	SEQ ID NO.
Variable Domains	VH1 D76N	-	64
	-	VL2	65
	-	VL2.1	66
	-	VL2.2	82
	-	VL2.3	83
Complete Sequences (without signal peptide)	VH1 D76N (G4wt)	-	67
	VH1 D76N (G4PRO)	-	68
	VH1 D76N (G2 wt)	-	69
	-	VL2-Ck	70
	-	VL2.1-Ck	71
	-	VL2.2-Ck	84
	-	VL2.3-Ck	85

[0144] In a preferred embodiment, the heavy chain sequences are deleted from their C-terminal lysine residue (as found in the original pConPlus vector series from Lonza: pConPlus γ 4 Δ K, pConPlus γ 4PRO Δ K & pConPlus γ 2 Δ K).

[0145] Moreover, the G4PRO heavy chain corresponds to a human IgG4 isotype carrying a mutation in the Hinge region to avoid formation of half-antibodies. This mutation is found in the parental pConPlus γ 4PRO Δ K from Lonza [Angal S, King DJ, Bodmer MW, Turner A, Lawson AD, Roberts G, Pedley B, Adair JR. A single amino acid substitution abolishes the heterogeneity of chimeric mouse/human (IgG4) antibody. Mol Immunol. (1993);30(1):105-108].

[0146] As an example, for the avoidance of doubt, the expression "VH1" is similar to the expressions "VH Variant 1", "VH variant 1", "VH Var 1" or "VH var 1".

[0147] It must be understood that the above exemplified VH / VL combinations are not limitative. The man skilled in the art could of course, without undue burden and without applying inventive skill, rearrange all the VH and VL disclosed in the present specification..

[0148] 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) a nucleic acid, DNA or RNA, coding for an antibody, or one of its functional fragments, according to the invention;
- b) a nucleic acid comprising a DNA sequence selecting from the group of sequences consisting of SEQ ID Nos. 14 to 19 and 41 to 45;
- c) a nucleic acid comprising a DNA sequence selecting from the group of sequences consisting of SEQ ID Nos. 20, 21, 46 and 47;
- d) the corresponding RNA nucleic acids of the nucleic acids as defined in b) or c);
- e) the complementary nucleic acids of the nucleic acids as defined in a), b) and c); and
- f) a nucleic acid of at least 18 nucleotides capable of hybridizing under conditions of high stringency with at least one of the CDRs of sequence SEQ ID Nos. 14 to 19 and 41 to 45.

[0149] Table 5 below summarizes the various nucleotide sequences concerning the antibodies of the invention.

Table 5 (wherein Mu. = murine)

Antibody	CDR numbering	Heavy chain	Light chain	SEQ ID NO.
515H7	IMGT		CDR-L1	14
			CDR-L2	15
			CDR-L3	16
		CDR-H1		17
		CDR-H2		18
		CDR-H3		19
	Kabat		CDR-L1	22
			CDR-L2	23
			CDR-L3	16
		CDR-H1		24
		CDR-H2		25
		CDR-H3		26
			Mu. variable domain	20
		Mu. variable domain		21

(continued)

Antibody	CDR numbering	Heavy chain	Light chain	SEQ ID NO.
301aE5	IMGT		CDR-L1	41
			CDR-L2	15
			CDR-L3	42
		CDR-H1		43
		CDR-H2		44
		CDR-H3		45
	Kabat		CDR-L1	48
			CDR-L2	49
			CDR-L3	50
		CDR-H1		51
		CDR-H2		52
		CDR-H3		53
			Mu. variable domain	46
		Mu. variable domain		47

[0150] 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.

[0151] 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.

[0152] "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.

[0153] 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.

[0154] 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).

[0155] The invention also encompasses an isolated nucleic acid molecule characterized in that it is selected among the following nucleic acids:

- a) a nucleic acid, DNA or RNA, coding for a humanized antibody heavy chain, or for a derived compound or functional fragment of same, according to the invention;
- b) a nucleic acid, DNA or RNA, coding for a humanized antibody light chain, or for a derived compound or functional fragment of same, according to the invention;
- 5 c) a nucleic acid, DNA or RNA, coding for a humanized antibody, or for a derived compound or functional fragment of same, according to the invention;
- d) a nucleic acid complementary to a nucleic acid as defined in a), b) or c);
- e) a nucleic acid of at least 18 nucleotides capable of hybridizing under highly stringent conditions with at least a heavy chain comprising the nucleic acid sequences SEQ ID No. 72 or 75 to 77;
- 10 f) a nucleic acid of at least 18 nucleotides capable of hybridizing under highly stringent conditions with at least a light chain comprising the nucleic acid sequences SEQ ID No. 73, 74, 86, 87 or 78, 79, 88, 89.

[0156] The table 6 thereafter summarizes the nucleotide sequences of the various heavy and light chains variable domains and full length (or complete), respectively, of the humanized antibody 515H7 according to the invention.

Table 6 (wherein Hz = humanized)

Antibody Hz515H7	Heavy chain	Light chain	SEQ ID NO.
Variable Domains	VH1 D76N	-	72
	-	VL2	73
	-	VL2.1	74
	-	VL2.2	86
	-	VL2.3	87
Complete Sequences (without signal peptide)	VH1 D76N (G4wt)	-	75
	VH1 D76N (G4PRO)	-	76
	VH1 D76N (G2 wt)	-	77
	-	VL2-Ck	78
	-	VL2.1-Ck	79
	-	VL2.2-Ck	88
	-	VL2.3-Ck	89

[0157] The table 7 herein-under summarizes the nucleotide sequences of the various heavy and light chains of the chimeric antibody 515H7 according to the invention.

Table 7 (wherein c = chimeric)

Antibody c515H7	Heavy chain	Light chain	SEQ ID NO.
Complete Sequences (without signal peptide)	cVH (G4wt)	-	60
	cVH (G4PRO)	-	61
	cVH (G2 wt)	-	62
	-	cVL-Ck	63

[0158] In other words, the invention deals with an isolated nucleic acid, characterized in that it is chosen from the following nucleic acids:

- a) a nucleic acid, DNA or RNA, coding for an antibody, or one of its functional fragments, according to the invention;
- b) a nucleic acid comprising a DNA sequence selecting from the group of CDRs sequences consisting of SEQ ID Nos. 14 to 19 and 41 to 45;
- c) a nucleic acid comprising a DNA sequence selecting from the group of heavy and light variable domains sequences consisting of SEQ ID Nos. 20, 21, 46, 47, 72, 73, 74, 86 and 87;
- d) a nucleic acid comprising a DNA sequence selecting from the group of heavy and light chains sequences consisting

of SEQ ID Nos. 60 to 63, 75 to 79, 88 and 89;

e) a nucleic acid comprising a DNA sequence consisting of SEQ ID No. 55;

f) the corresponding RNA nucleic acids of the nucleic acids as defined in b), c), d) or e);

g) the complementary nucleic acids of the nucleic acids as defined in a), b), c), d) and e); and

h) a nucleic acid of at least 18 nucleotides capable of hybridizing under conditions of high stringency with at least one of the CDRs of sequence SEQ ID Nos. 14 to 19 and 41 to 45.

[0159] The invention also relates to a vector comprising a nucleic acid as described in the invention.

[0160] The invention notably targets cloning and/or expression vectors that contain such a nucleotide sequence.

[0161] 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.

[0162] 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.

[0163] 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.

[0164] The invention also comprises host cells transformed by or comprising a vector as described in the present invention.

[0165] 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.

[0166] The invention also relates to animals, other than man, that have a transformed cell according to the invention.

[0167] 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.

[0168] 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.

[0169] 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.

[0170] 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.

[0171] The antibodies, or the functional fragments of same, likely to be obtained by the method of the invention are also comprised in the present invention.

[0172] According to still another aspect, the present invention relates to antibodies as described above, characterized in that they are, in addition, capable of specifically binding to a human chemokine family receptor and/or capable of specifically inhibiting X4-tropic HIV replication.

[0173] According to still another aspect, the present invention relates to antibodies as described above, characterized in that they are, in addition, capable of specifically binding to a human chemokine family receptor and/or capable of specifically inhibiting X4/R5-tropic HIV replication.

[0174] The present disclosure also relates to antibodies, or their derived compounds or functional fragments, consisting of antibody that are bispecific in the sense that they comprise a second motif capable of interacting with any receptor implicated in HIV cell entry, such as, for example, CCR5, CD4, CXCR4 (other than the antibody of the present invention, i.e. targeting another epitope) or CCR3, CCR2, CCR8, CXCR6, CXCR7, CX3CR1.

[0175] 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 target several molecules on the surface of 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).

[0176] 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.

[0177] 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.

[0178] Preferably, the bispecific antibodies are bivalent or tetravalent antibodies.

[0179] Lastly, the present invention relates to the antibodies described above, or one of their functional fragments thereof, as a medicament.

[0180] 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 functional fragments or derivatives. Preferably, said antibody is supplemented by an excipient and/or a pharmaceutically acceptable carrier.

[0181] The invention also relates to the composition as above described as a medicament.

[0182] In a particular aspect of the invention, the antibody, or one of its functional fragments, inhibits HIV-1 KON primary isolate replication in PBMC with an IC₉₀ of at least 5 µg/ml, preferably at least 10 µg/ml.

[0183] The present invention also comprises the use of an antibody or the composition according to the invention for the preparation of a drug and/or medicament for the prevention or the treatment of HIV infection.

[0184] More particularly, as a non limitative example, said HIV infection is a X4-tropic HIV infection.

[0185] In another embodiment, as a non limitative example, said HIV infectious is a X4/R5-tropic HIV infection.

[0186] The present invention also relates to the use of an antibody, or a functional fragment or derivative of same, preferably humanized, and/or of a composition according to the invention for the preparation of a drug for inhibiting HIV replication. Generally, the present invention relates to the use of an antibody, or a functional fragment or derivative of same, preferably humanized, and/or of a composition, for the preparation of a drug for HIV disease prevention or treatment.

[0187] 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.

[0188] Preferably, such compounds will be administered by systemic route, notably by intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, intravaginal 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.

[0189] 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.

[0190] The invention also relates to a composition comprising, in addition, as a combination product for use in a simultaneous, separated or extended fashion, an anti-HIV antibody or an anti-HIV cell entry antibody or an anti-HIV replication antibody other than an antibody directed against CXCR4.

[0191] According to still another embodiment, the present invention also relates to a pharmaceutical composition as described above that comprises at least a second anti-HIV compound selected among the compounds capable of specifically inhibiting HIV entry and/or replication such as anti-CCR5, anti-CD4 compounds and anti-CXCR4 compounds other than those described in this invention, or any other anti-HIV compound known to a person skilled in the art.

[0192] Another embodiment complementary to the invention consists of a composition as described above comprised of, in addition, as a combination or conjugation product for simultaneous, separated or extended use, an anti-HIV compound.

[0193] "Simultaneous use" means the administration of both compounds of the composition comprised in a single

dosage form.

[0194] "Separated use" means administration, at the same time, of both compounds of the composition, comprised in distinct dosage forms.

[0195] "Extended use" means the successive administration of both compounds of the composition, each comprised in a distinct dosage form.

[0196] Generally, the composition according to the invention considerably increases HIV treatment effectiveness. In other words, the therapeutic effect of the antibody of the invention is enhanced in an unexpected way by the administration of an anti-HIV 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 anti-HIV agent. Moreover, this composition makes it possible to achieve the expected therapeutic effect more quickly.

[0197] "Therapeutic anti-HIV agent" means a substance which, when it is administered to a patient, treats or prevents the replication of HIV in the patient. Non-limiting examples of such agents include "antiretroviral drugs such as HIV protease inhibitors (PI), nucleoside /nucleotide HIV reverse-transcriptase inhibitors (NRTI/NtRTI) non-nucleoside HIV reverse-transcriptase inhibitors (NNRTI), HIV entry inhibitors, HIV integrase inhibitors".

[0198] Such agents, for example, are cited in VIDAL, on the pages devoted to compounds related to "anti-HIV compounds"; the anti-HIV compounds cited by reference to this document are cited herein as non limitative preferred anti-HIV agents.

[0199] HIV protease inhibitor refers to any substance that can inhibit HIV protease activity. Examples of such HIV protease inhibitors include, but are not limited to Saquinavir mesylate or SQV (Invirase®), Indinavir or IDV (Crixivan®), Ritonavir or RTV (Norvir®), Nelfinavir or NFV (Viracept®), Amprenavir (Agenerase®, Prozei®), Lopinavir/ritonavir or LPV/r (Kaletra®, Aluvia®), Atazanavir or ATV (Reyataz®, Zrivada®), Fosamprenavir or FPV (Lexiva®, Telzir®), Tipranavir or TPV (Aptivus®), Darunavir or DRV (Prezista®)

[0200] HIV Nucleoside or nucleotide reverse-transcriptase inhibitor (NRTI) refers to a substance which is a nucleoside or nucleotide analogue that blocks reverse-transcription of HIV RNA. Examples of NRTI include, but are not limited to Zidovudine or AZT, ZDV (Retrovir/combivir/trixivir®), Didanosine or ddi (Videx®), Zalcitabine (HIVID®), Stavudine or d4T (Zerit®), Lamivudine or 3TC (Epivir/combivir/epzicom/trixivir®), Abacavir or ABC (Ziagen/trixivir/epzicom®), Tenofovir disoproxil fumarate or TDF (Viread/atrima/truvada®), Emtricitabine or FTC (Emtriva/atrima/truvada®)

[0201] Non-nucleoside HIV reverse-transcriptase inhibitor (NNRTI) refers to a substance which is not a nucleoside or nucleotide analogue that blocks reverse-transcription of HIV RNA. Examples of NNRTI include, but are not limited to Nevirapine or NVP (Viramune®), Efavirenz or EFV (Sustiva/atrima®, Stocrin®), Delavirdine or DLV (Rescriptor®) and Etravirine or ETR (Intelence®).

[0202] HIV entry inhibitor refers to a substance that block HIV cell entry. Examples of HIV entry inhibitors include, but are not limited to Enfuvirtide or T20 (Fuzeon®), Maraviroc or MVC (Celsentri®, Celzentry®).

[0203] HIV integrase inhibitor refers to a substance that inhibits HIV integrase activity. Example of integrase inhibitor includes, but is not limited to Raltegravir or RAL (Isentress®).

[0204] Such agents, for example, are also compounds belonging to the same classes of drugs described in VIDAL, which are currently in clinical trials such as but not limited to Vicriviroc, PRO140, TNX-355, AMD070, Racivir, Apricitabine, Elvucitabine, Flosalvudine, Rilpivirine, Elvitegravir.

[0205] Such agents, for example, are also compounds belonging to other potential classes of drugs such as but not limited to maturation inhibitors (Bevirimat), glycoside analogues of β -galactosyl-ceramide, carbohydrate-binding agents, RNaseH inhibitors, HIV gene expression inhibitors, stimulators of HIV release from latent T cells (valproic acid ...).

[0206] In a particularly preferred embodiment, said composition of the invention as a combination product is characterized in that said anti-HIV agent is bound chemically to said antibody for use simultaneously.

[0207] In order to facilitate binding between said anti-HIV agent and said antibody, 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 anti-HIV 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.

[0208] Also preferably, said antibody forming said conjugate is selected among its functional fragments, notably fragments that have lost their Fc component, such as scFv fragments.

[0209] The invention also relates to a composition as a combination product or to an anti-CXCR4 Mab/anti-HIV drug conjugate, according to the invention, used as drug.

[0210] Preferably, said composition as a combination product or said conjugate will be supplemented by an excipient and/or a pharmaceutical vehicle.

[0211] 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 HIV replication.

[0212] As previously demonstrated, CXCR4 Mabs 515H7 and 301 aE5 have strong activities against HIV-1 replication

in PBMC, so such antibodies could be used in screening assays for identification of CXCR4 antagonist antiviral agents to treat HIV-1 infection. In the first step of these assays, cells expressing CXCR4 are incubated with Mabs 515H7 and/or 301aE5 and then molecules can be evaluated for their potential to inhibit Mabs 515H7 and/or 301aE5 binding. Cells used in this type of assays can be transfected cell lines such as CHO-CXCR4, NIH3T3-CXCR4 or CXCR4 transfected human cell lines such as U373-MAGI-CXCR4, human cell lines expressing CXCR4 such as NALM6 or primary cells such as PBMC. The method used to screen antagonists of CXCR4 inhibiting Mabs 515H7 and/or 301aE5 binding on CXCR4 expressing cells can be cell-based competitive enzyme-linked immunosorbent Assay (ELISA) as described by Zhao Q. et al. (AIDS Research And Human Retroviruses, 2003, 19, pp947-955) or protocols using Fluorescence-Activated cell Sorting (FACS) such as described by Juarez J. et al. (Leukemia 2003, 17, pp1294-1300).

[0213] Thus, in a particular aspect of the invention, it is considered a process for the screening and/or the identification of molecules as CXCR4 antagonist antiviral agents comprising the steps of:

- a) selecting cells expressing CXCR4,
- b) incubating said cells with an antibody, or one of its functional fragments, of the invention, and
- c) evaluating the tested molecules for their potential inhibition of the binding between the antibody, or one of its functional fragments, to CXCR4, and
- d) selecting molecules capable of said inhibition.

[0214] In another particular embodiment, the following step e) can be added:

- e) testing these molecules in a HIV-1 replication assay.

[0215] Other characteristics and advantages of the invention appear further in the description with the examples and figures whose legends are presented below.

FIGURE LEGENDS

[0216]

Figures 1A and 1B show the gating strategy for CXCR4 expression on monocytes and lymphocytes.

Figure 1A: T cell staining with CD3-PE antibody.

Figure 1B: monocytes staining with CD14-PE antibody.

Figures 2A and 2B show the binding of anti-CXCR4 Mabs 515H7 and 301aE5 on monocytes and T lymphocytes. Figures 3A and 3B show the modulation of CXCR4 receptor dimer by SDF-1 and by anti-CXCR4 Mabs 515H7 and 301aE5, respectively, via a bioluminescence resonance energy transfer (BRET) approach in HEK293 cells. Figures 4A and 4B and Figure 5 show the ability of anti-CXCR4 Mab 515H7 and 301aE5 to inhibit HIV-1 isolate KON (X4 virus) replication in human PBMC.

Figures 6A, 6B and 6C show the inhibition of SDF-1-induced calcium release in CHO-CXCR4 cells by Mabs 515H7 (figure 6A), 301aE5 (figure 6B) and c515H7 (figure 6C).

Figures 7 and 8 show the ability of anti-CXCR4 Mabs 515H7, c515H7 and 301aE5 to inhibit HIV-1 X4 virus primary isolates MN (figure 7) and 92UG024 (figure 8) replication in human PBMC.

Figure 9 shows the ability of anti-CXCR4 Mabs 515H7, c515H7 and 301aE5 to inhibit HIV-1 X4 virus primary isolates KON, MN and 92UGO24 replication in human PBMC.

Figures 10 and 11 show the ability of anti-CXCR4 Mabs 515H7, c515H7 and 301aE5 to inhibit the dual HIV-1 X4/R5 virus primary isolate 89.6 replication in human PBMC.

Figure 12 shows the beneficial effect of combining Mab c515H7 with Maraviroc to inhibit HIV-1 primary isolate 89.6 (dual X4/R5 virus) replication in human PBMC.

Figure 13 shows the beneficial effect of combining Mab c515H7 with Maraviroc to inhibit HIV-1 primary isolate UG93067 (dual X4/R5 virus) replication in human PBMC.

Figure 14 shows the ability of anti-CXCR4 Mabs 515H7, c515H7 and 301aE5 to inhibit HIV-1 X4 virus primary isolate KON replication in PBMC.

Figure 15 illustrates the binding specificity of the c515H7 Mab by FACS analysis.

Figure 16 illustrates the effect of c515H7 Mab on CXCR4 homodimer, by bioluminescence resonance energy transfer (BRET) approach.

Figure 17: Amino acid sequences alignment of 515H7 heavy chain variable domain with the human germline IGHV3-49*04 and IGHJ4*01. The 515H7 VH amino acid sequence is aligned with the selected human acceptor

framework sequences. VH Var1 (VH1) sequences correspond to the humanized variants of 515H7 VH domains. The single back mutation in position 76 is depicted in bold.

Figure 18: Amino acid sequences alignment of 515H7 light chain with the human germline IGKV4-1*01 and IGKJ1*01. The 515H7 VL amino acid sequence is aligned with the selected human acceptor framework sequences. VL Var2.1, Var2.2 and Var2.3 sequences correspond to implemented humanized variants of the humanized 515H7 VL Var2, with mutated residues in bold. Var2.1 and Var2.2 carry 4 more humanized residues whereas Var2.3 contains 5 more human residues.

Figure 19: Cross blocking of the biotinylated murine antibody 515H7 by the chimeric 515H7 and different variants of the humanized 515H7. The activity of the humanized variants of 515H7 (hz515H7) to cross block the parental murine antibody 515H7 was evaluated by flow cytometry using CXCR4 transfected NIH3T3 cells. The activity of the humanized variants was compared to the chimeric 515H7. The cross blocking activity of the variant VH1 combined with the chimeric VL (cVL) was very similar to that of the chimeric (A). No reduction in the activity of VH variant 1 (VH1, the variant with no back mutations) was determined when combined with variant 2 of VL (B).

Figure 20: Inhibition of the biotinylated SDF-1 binding by the chimeric 515H7 and different variants of the humanized 515H7. The capacity of the humanized variant of 515H7 (hz515H7) to inhibit SDF-1 binding was evaluated by flow cytometry using the cell line RAMOS. The inhibition capacity of the humanized variants was compared to the chimeric 515H7. The humanized variant hz515H7 VH1 D76N VL2 has a similar capacity to inhibit SDF-1 binding as the chimeric antibody. The humanized antibody fragment hz515 VH1 VL2 was fully active in inhibiting the binding of SDF-1 to RAMOS cells.

Figure 21 shows humanized 515H7 Mabs (hz515H7 VH1 D76N VL2, hz515H7 VH1 D76N VL2.1, hz515H7 VH1 D76N VL2.2 and hz515H7 VH1 D76N VL2.3) specific binding to CXCR4 on NIH3T3-CXCR4.

Figure 22 shows the effect of humanized 515H7 Mabs (hz515H7 VH1 D76N VL2, hz515H7 VH1 D76N VL2.1, hz515H7 VH1 D76N VL2.2 and hz515H7 VH1 D76N VL2.3) on CXCR4 homodimer, by bioluminescence resonance energy transfer (BRET) approach.

EXAMPLES

Example 1: Generation of monoclonal antibodies (Mabs) against human CXCR4

[0217] 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. 6-16 weeks of age mice 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 below) 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

[0218] 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 5 µg equivalent peptide/mL, 100 µL/well incubated at 4°C overnight, then blocked with 250 µL/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 H₂SO₄. Mice that developed the highest titers of anti-CXCR4 antibodies were used for antibody generation.

- Generation of hybridomas producing Mabs to CXCR4

[0219] 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 10⁵ /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 2: Characterization of anti-CXCR4 Mab 515H7 and 301aE5 binding specificity (NIH3T3-CXCR4 transfectant) by FACS analysis

[0220] In this experiment, the specific binding to human CXCR4 (hCXCR4) of anti-CXCR4 Mabs 515H7 and 301aE5 was examined by FACS analysis. NIH3T3 and NIH3T3-hCXCR4 transfected cells were incubated with 10 µg/ml of monoclonal antibodies 515H7 and 301aE5. 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 8 which shows [Mean Fluorescence Intensity (MFI) obtained by FACS] that anti-CXCR4 Mabs 515H7 and 301aE5 bind specifically to human CXCR4-NIH3T3 transfected cell line whereas there was no recognition on the parent NIH3T3 cells.

Table 8

	NIH3T3 (MFI)	NIH3T3-CXCR4 (MFI)
Clone 515H7 (10 µg/ml)	16	2752
Clone 301aE5 (10 µg/ml)	21	1367

Example 3: Characterization of anti-CXCR4 Mab 515H7 and 301aE5 binding to peripheral blood mononuclear cells (PBMC) by FACS analysis

[0221] Blood was collected as buffy coat from healthy donor. 100 µl of whole blood was incubated with anti-human CXCR4 antibodies (clones 515H7 and 301aE5) at the indicated concentration for 20 minutes at 4°C. Blood was washed three times in PBS-BSA 1%-NaN₃ 0.01% and incubated with goat anti-human Alexa 488 IgG diluted 1:500 (Invitrogen) for 20 minutes at 4°C. Cells were then washed and incubated with CD14-PE (Caltag) or CD3-PE (Caltag) for 10 minutes at 4°C and washed three times. Red blood cells were lysed with High-Yield lyse solution (Caltag) for 10 minutes at room temperature. Cells were analyzed immediately using Facscalibur (Becton-Dickinson). CXCR4 expression on monocytes was done on CD14 positive cells and CXCR4 expression on T cell was done on CD3 positives cells (figure 1). Results are expressed in Antigen Binding Capacity (ABC).

[0222] As shown in figures 2A and 2B, the anti-human CXCR4 clones 515H7 and 301aE5 stained both T lymphocytes (Figure 2A) and monocytes (Figure 2B) indicating that 515H7 and 301aE5 Mabs are able to recognize the native form of CXCR4 expressed at the cell surface of monocytes and T lymphocytes.

Example 4: Effect of 515H7 and 301aE5 Mabs on CXCR4 homodimer, by bioluminescence resonance energy transfer (BRET) approach

[0223] This functional assay allows to evaluate the conformational changes induced upon SDF-1 and/or 515H7 Mab binding to CXCR4 receptor at the level of CXCR4 homodimer.

[0224] Expression vectors for 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 homodimerization. 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 CO₂ 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).

[0225] Calculation of BRET ratio was performed as previously described (Angers et al., 2000): $[(\text{emission}_{530 \text{ nm}}) - (\text{emission}_{485 \text{ nm}}) \times \text{Cf}] / (\text{emission}_{485 \text{ nm}})$, where $\text{Cf} = (\text{emission}_{530 \text{ nm}}) / (\text{emission}_{485 \text{ nm}})$ 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.

[0226] 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 3A and B). 515H7 and 301aE5 Mabs were able to modulate SDF-1-induced conformational changes for CXCR4 homo-dimers (69 % inhibition of SDF-1-induced BRET increase for 515H7 and 301aE5, Figures 3A and B). 515H7 and 301aE5 Mabs were also able to modulate by themselves CXCR4/CXCR4 spatial proximity, indicating an influence of 515H7 and 301aE5 Mabs on CXCR4/CXCR4 homodimer conformation. (Figures 3A and 3B).

Example 5: Inhibition of HIV-1 primary isolate KON (X4 virus) replication in human PBMC by anti-CXCR4 Mabs 515H7 and 301aE5

[0227] PBMC from normal donors seronegative for HIV-1 were isolated from buffy coats or cytapheeresis by Ficoll-Hypaque gradient centrifugation. PBMC were activated in the presence of PHA in RPMI 1640 cell culture medium containing 25 mM HEPES, 5 ml penicillin (10000 U/ml) - streptomycin (10000 µg/ml) 2 mM L-glutamine, supplemented with 10% heat-inactivated FCS and were used as cellular targets in a single cycle neutralization assay. HIV-1 replication in primary human PBMC was performed by analyzing intracellular staining of viral p24 antigen by FACS analysis. Briefly, 25 µl per well of various dilutions of Mabs 515H7, 301aE5 and 12G5 (R&D Systems) or culture medium as control (RPMI 1640, 10% FCS, 0.1% IL-2), were incubated for 1 h at 37°C with 25 µl per well of HIV-1 KON X4 primary isolate dilution, in duplicate. PHA-activated human PBMC (25 µl/well) at 20×10^6 cells/ml were added to the Mab/virus mixture in a 96-well plate (U-bottom, Costar 3599) and cultured for 24 to 36 hours at 37°C in RPMI 1640 10% FCS and 0.1% IL-2. Control consisting of uninfected PBMC in medium without Mab was introduced. To detect HIV-infected PBMC, intracellular staining of viral p24 antigen was performed and analyzed by flow cytometry. Cells were fixed and permeabilized using the Cytotfix/Cytoperm kit (Becton Dickinson) according to the manufacturer protocols and stained with a fluorescent anti-p24 Mab (clone KC57 - Coulter Beckman) used at a 1/160 dilution incubated for 10 min at 4°C in the dark. After washing in PBS-3% FCS medium, PBMC were diluted in PBS before flow cytometry analysis. The percentage of p24-positive cells in the different samples was determined by gating 20,000 events on a living cell population. The living cell subsets were analyzed for p24 expression relative to background staining of uninfected cells. The p24 antigen-positive value was obtained after subtraction of background events in mock-infected cells. The percentage of neutralization was defined as the reduction of p24-positive cells compared with control infected wells with no Mab. The neutralizing titer was defined as the dilution of Mab that allows a 90% decrease in the percentage of infected cells. The anti-CXCR4 Mabs 515H7 and 301aE5 were compared to 12G5 Mab known as the anti-CXCR4 Mab of reference for HIV application. As shown in Figures 4A and B and 5, the anti-CXCR4 Mabs 515H7 and 301aE5 are able to inhibit HIV-1 KON primary isolate replication in PBMC with an IC_{90} of 10 µg/ml (66 nM) and 150 µg/ml (1 µM), respectively, whereas 12G5 Mab failed to inhibit HIV-1 KON primary isolate replication in PBMC (Figure 4A).

Example 6: CXCR4 receptor-mediated mobilization of intracellular calcium stores

[0228] 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.

[0229] CHO-K1 cells, stably and constitutively expressing human CXCR4 receptor were obtained upon transfection of naive 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]. Cells 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 subtracting 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).

[0230] SDF1 (100 nM) induced a rapid and strong release of intracellular calcium in recombinant CHO/CXCR4, whereas no fluorescence signal was detected in naive CHO-K1 cells. The maximal intensity reached > 140 % over basal fluorescence and was observed at about 40 sec. upon stimulation by SDF-1 (Figures 6A, 6B and 6C). Mabs 515H7 (133 nM) (Figure 6A) and c515H7 (133nM) (Figure 6C) yielded a strong inhibition of the SDF-1 (100 nM)-induced calcium signal. Mab 301aE5 (133 nM) (Figure 6B) yielded a partial inhibition of the SDF-1 (100 nM)-induced calcium signal.

Example 7: Inhibition of HIV-1 primary isolates KON, MN and 92UG024 (X4 viruses) replication in human PBMC by anti-CXCR4 Mabs 515H7, c515H7 and 301aE5

Single cycle neutralization assay.

[0231] This assay is performed in 36 h using primary isolates KON, MN and 92UG024 concentrated and diluted accordingly to allow the detection 2% infected CD4 T lymphocytes after 2 days of infection.

[0232] Twenty-five microliters of various dilutions of Mabs 515H7, c515H7 and 301aE5 were incubated for 1 h at 37°C with 25 µl of virus. Human PBMC (25 µl) at 20 x 10⁶ cells/ml were added to the Mab/virus mixture in a 96-well plate (U-bottom, Costar 3599) and cultured for 36 h in RPMI 1640 10% FCS and 20 U/ml IL-2 (R&D Systems, Minneapolis, MN).

[0233] After 2 days of culture, HIV-infected lymphocytes were detected by intracellular staining of viral p24 Ag. Cells were fixed and permeabilized using both Cytofix/Cytoperm and Perm/Wash kits (BD Biosciences) according to the manufacturer and stained with a fluorescent anti-p24 Mab (FITC- or PE-anti-p24, clone KC57; Beckman Coulter/Immuno-tech, Hialeah, FL) used at a 1/160 dilution in Perm/Wash solution added for 15 min at 4°C. After washing in PBS with 3% FBS, PBMC were diluted in 300 µl of PBS before flow cytometry analysis (LSRII; BD Biosciences) with DIVA software (BD Biosciences). The percentage of p24-positive cells in the different samples was determined by gating 20,000 events on a living cell population identified by forward- and side-scatter parameters. The living cell subsets were analyzed with the live/dead solution kit (Invitrogen). The p24 Ag-positive value was obtained after subtraction of background events in mock-infected cells.

[0234] The percent of neutralization was defined as the reduction of p24-positive cells compared with control infected wells with no Mab. The neutralizing titer was defined as the concentration of antibody (interpolated between successive dilutions performed in triplicate) that allows a 90% decrease in the percentage of infected cells.

[0235] As shown in Figures 7, 8 and 9, the anti-CXCR4 Mabs 515H7, c515H7 and 301aE5 are able to inhibit HIV-1 X4 MN, KON and 92UG024 primary isolates replication in PBMC. Results of ICs (in µg/ml) are summarized in table 9.

Table 9

	KON			MN			92UG024		
	% inhibition of infected cells			% inhibition of infected cells			% inhibition of infected cells		
	90	80	50	90	80	50	90	80	50
301aE5	>150	>150	150	>150	150	1	>150	150	50
515H7	15	8	1	10	1.5	<1.5	10	3	0.5
c515H7	20	10	1.5	15	1	<1.5	15	3	<1.5

Example 8: Inhibition of HIV-1 primary isolate 89.6 (dual X4/R5 virus) replication in human PBMC by anti-CXCR4 Mabs 515H7, c515H7 and 301aE5

Single cycle neutralization assay

[0236] This assay is performed in 36 h using primary isolate 89.6 concentrated and diluted accordingly to allow the detection 2% infected CD4 T lymphocytes after 2 days of infection.

[0237] Twenty-five microliters of various dilutions of Mabs 515H7, c515H7 and 301aE5 were incubated for 1 h at 37°C with 25 µl of virus. Human PBMC (25 µl) at 20 x 10⁶ cells/ml were added to the Mab/virus mixture in a 96-well plate (U-bottom, Costar 3599) and cultured for 36 h in RPMI 1640 10% FCS and 20 U/ml IL-2 (R&D Systems, Minneapolis, MN).

[0238] After 2 days of culture, HIV-infected lymphocytes were detected by intracellular staining of viral p24 Ag. Cells were fixed and permeabilized using both Cytofix/Cytoperm and Perm/Wash kits (BD Biosciences) according to the manufacturer and stained with a fluorescent anti-p24 Mab (FITC- or PE-anti-p24, clone KC57; Beckman Coulter/Immuno-tech, Hialeah, FL) used at a 1/160 dilution in Perm/Wash solution added for 15 min at 4°C. After washing in PBS with 3% FBS, PBMC were diluted in 300 µl of PBS before flow cytometry analysis (LSRII; BD Biosciences) with DIVA software (BD Biosciences). The percentage of p24-positive cells in the different samples was determined by gating 20,000 events on a living cell population identified by forward- and side-scatter parameters. The living cell subsets were analyzed with the live/dead solution kit (Invitrogen). The p24 Ag-positive value was obtained after subtraction of background events in mock-infected cells.

[0239] The percent of neutralization was defined as the reduction of p24-positive cells compared with control infected wells with no Mab. The neutralizing titer was defined as the concentration of antibody (interpolated between successive

dilutions performed in triplicate) that allows a 90% decrease in the percentage of infected cells.

[0240] As shown in Figures 10 and 11, the anti-CXCR4 Mabs 515H7, c515H7 and 301aE5 are able to inhibit HIV-1 89.6 primary isolate replication in PBMC. Results of ICs (in $\mu\text{g/ml}$) are summarized in table 10.

Table 10

	89.6		
	% inhibition of infected cells		
	90	80	50
301aE5	>150	150	20
515H7	15	1.5	<1.5
c515H7	10	1.5	<1.5

Example 9: Inhibition of HIV-1 primary isolates 89.6 and UG93067 (dual X4/R5 viruses) replication in human PBMC by anti-CXCR4 Mab c515H7 combined with the anti-CCR5 molecule Maraviroc

Neutralization assay, analyzing multiple round of HIV primary isolate replication on primary PBMC:

[0241] This assay that combines serial dilutions of c515H7 Mab or Maraviroc or combination of both with serial dilutions of virus, analyzes multiple rounds of infection on PBMC (peripheral blood mononuclear cells). Briefly, quadruplicate 25- μl aliquots of serial dilutions (twofold) of c515H7 Mabs or Maraviroc or combination of both were each incubated with 25 μl of serial dilutions of virus in prehydrated 96-well filter plates (1.25- μm pore size, Durapor Dv; Millipore, Molsheim, France). A control titration of the virus (25 μl of RPMI replacing the diluted c515H7 Mabs or Maraviroc) was performed on the same plate as the titrations in the presence of dilutions of the c515H7 Mabs or Maraviroc or combination of both. After 1 h at 37°C, 25 μl of PHA-stimulated PBMC at a concentration of 4×10^6 cells/ml (pool of PHA activated PBMC from five healthy donors) was added to achieve a 75- μl final culture volume of RPMI, 10% fetal calf serum (FCS), and 20 IU of interleukin-2 (IL-2) per ml (R&D System). After 24 h at 37°C, 100 μl of the same culture medium was added. Two washings (200 μl of RPMI each) were performed by filtration on day 4 to remove the c515H7 Mabs and Maraviroc, and 200 μl of fresh culture medium was added. On day 7, the presence of p24 in the culture supernatants was measured by ELISA and compared to that of the negative controls (cultures infected with dilutions of virus and maintained in the presence of 10^{-6} M zidovudine [AZT]) to determine positive wells. Quadruplicate wells were used to determine the viral titer (50% tissue culture infective dose [TCID₅₀]) in the absence (V_0) and in the presence (V_n) of each dilution of the c515H7 Mabs or Maraviroc or combination of both. The neutralizing titer was defined as the dilution of c515H7 Mabs or Maraviroc or combination of both resulting in a 90% decrease of the viral titer ($V_n/V_0 = 0.1$).

[0242] As shown in Figure 12, the dual tropic X4R5 virus 89.6 replication was inhibited by c515H7 Mabs with an IC₉₀ of 2 $\mu\text{g/ml}$ (Figure 12). Maraviroc at 50 $\mu\text{g/ml}$ did not reach the IC₉₀ inhibitory activity (Figure 12). Moreover, addition of Maraviroc at 2 $\mu\text{g/ml}$ to antibody 515H7 augmented the inhibitory activity of the c515H7 Mabs with an IC₉₀ of 0.2 $\mu\text{g/ml}$ (Figure 12).

[0243] The beneficial effect of c515H7 Mabs and Maraviroc combination was assessed using various dilutions of the two molecules and another dual tropic virus UG93067. As shown in Figure 13, the inhibitory activity of Mabs c515H7 and Maraviroc was similar. These results suggest that the capacity of virus UG93067 to use either CCR5 or CXCR4 receptor was comparable. Using the UG93067 virus, a better activity could be evidenced, only the combination of these X4 (c515H7 Mabs) and R5 (Maraviroc) inhibitors (10 $\mu\text{g/ml}$ of each) allowed a 90 % reduction of virus titer (Figure 13).

Example 10: Production of anti-CXCR4 chimeric Mab c515H7

[0244] Chimeric format of murine 515H7 Mab was designed: it corresponds to the light and heavy chain variable domains of the murine antibody of interest, genetically fused to human Ckappa and IgG1/IgG2/IgG4 constant domains. The recombinant Mab were produced upon transient transfection by using the HEK293/EBNA system with a pCEP4 expression vector (Invitrogen, US).

[0245] Respective amino acid and nucleotide sequences were described above in the specification. Moreover, as the above table 3 discloses the sequences of the IgG2 and 4 isotypes (which are preferred isotypes), it can also be mentioned here the sequence of the heavy chain of the IgG1 isotype, i.e. c515H7 VH (G1wt) which corresponds to the amino acid sequence SEQ ID No. 80 and the nucleotide sequence SEQ ID No.81.

[0246] The entire nucleotide sequences corresponding to the variable domains of 515H7 Mab 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/IgG2/IgG4 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).

[0247] 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 $2 \cdot 10^6$ 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 μ g/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 10^6 cells/ml. Cultivation process was monitored on the basis of cell viability and Mab production. Typically, cultures were maintained for 4 to 5 days. Mab was purified using a conventional chromatography approach on a Protein A resin (GE Healthcare, US). Mab was produced at levels suitable with functional evaluations. Productivity levels are typically ranging between 6 and 15 mg/l of purified Mab.

Example 11: Characterization of anti-CXCR4 chimeric Mab c515H7 binding specificity by FACS analysis

[0248] In this experiment, specific binding to human CXCR4 of anti-CXCR4 chimeric Mab c515H7 was examined by FACS analysis.

[0249] NIH3T3-hCXCR4 transfected cells were incubated with a dose range of monoclonal antibody c515H7 from 0 μ g/ml to 10 μ g/ml. 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 this binding study are provided in Figure 15 which shows that anti-CXCR4 chimeric Mab c515H7 bound specifically to human CXCR4-NIH3T3 transfected cell line. No binding was detected to NIH3T3 wt cells (data not shown).

Example 12: Effect of c515H7 Mab on CXCR4 homodimer, by bioluminescence resonance energy transfer (BRET) approach

[0250] This functional assay allows to evaluate the conformational changes induced upon SDF-1 and/or c515H7 Mab binding to CXCR4 receptor at the level of CXCR4 homodimer.

[0251] Expression vectors for 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 homodimerization. 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 CO₂ 5 % in order to allow cell attachment to the plate. Cells were then starved with 200 μ l 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 μ M with or without SDF-1 100 nM in a final volume of 50 μ l. 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).

[0252] Calculation of BRET ratio was performed as previously described (Angers et al., 2000): $[(\text{emission}_{530 \text{ nm}}) - (\text{emission}_{485 \text{ nm}}) \times \text{Cf}] / (\text{emission}_{485 \text{ nm}})$, where $\text{Cf} = (\text{emission}_{530 \text{ nm}}) / (\text{emission}_{485 \text{ nm}})$ 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.

[0253] 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 16). Mab c515H7 was able to modulate SDF-1-induced conformational changes for CXCR4 homo-dimers (96% inhibition of SDF-1-induced BRET increase, Figure 16). Mab c515H7 was also able to modulate by itself CXCR4/CXCR4 spatial proximity, indicating an influence of this Mab on CXCR4/CXCR4 homodimer conformation (Figure 16).

Example 13: *In vitro* evaluation of Mab 515H7 anti-HIV-1 activity using GFP-transduced human osteosarcoma (GHOST) cells expressing CD4 and CXCR4 or CCR5.

[0254] In order to determine the specificity of CXCR4 515H7 Mab, we evaluated the anti-HIV-1 activity of this Mab using GHOST cells expressing CD4 and CXCR4 or CCR5.

[0255] This assay is performed in 48 h using either the X4 HIV-1 LAI virus (with Ghost cells expressing CXCR4) or the R5 HIV-1 BaL virus (with Ghost cells expressing CCR5). 500 μ l of Ghost cells were plated ($2.5 \cdot 10^5$ cells/ml) for 24h in Dulbecco culture medium supplemented with 10% FCS. Various dilutions of Mab 515H7 were incubated for 1 h at 37°C and then diluted HIV-1 LAI virus (1/10) and HIV-1 BaL virus (1/7) were added to the cells for 48h. Cells were submitted to trypsin and washed with PBS 1X. 300 μ L of 1.5% paraformaldehyde were added to the cell pellets for 2h at +4°C in the dark, in order to fix the cells and to inactivate viruses. GFP-positive cells were analyzed by flow cytometry and inhibition of HIV-1 infection was calculated.

[0256] The percent of inhibition of infected cells was defined as the compared with control infected wells with no Mab. Results of ICs (in μ g/ml) are summarized in table 11, the anti-CXCR4 Mab 515H7 was able to inhibit HIV-1 X4 Lai virus infection in CXCR4 expressing Ghost cells but was totally inactive in inhibiting HIV-1 R5 BaL virus infection in CCR5 expressing Ghost cells.

Table 11

	GHOST CXCR4/ LAI virus			GHOST CCR5/ BaL virus		
	% inhibition of infected cells			% inhibition of infected cells		
	90	80	50	90	80	50
515H7	0.5	0.1	0.08	>75	>75	>75

Example 14: humanization of 515H7 anti-CXCR4 murine antibody and generation of a fragment of said h515H7

- General procedure

[0257] Humanization of 515H7 anti-CXCR4 antibody was performed by applying the global rules of CDR-grafting. Immunogenetic analysis and definition of CDR and framework (FR) regions were performed by applying the IMGT unique numbering scheme as well as the IMGT libraries and tools (Lefranc, 1997 - www.imgt.org).

[0258] Binding of humanized variants of 515H7 was determined on a NIH3T3 cell line stably transfected with human CXCR4. The binding activity was evaluated by a competition assay with the biotinylated mouse antibody. In a second attempt, humanized antibodies were evaluated for their ability to inhibit binding of biotinylated SDF-1 to RAMOS cells. RAMOS cells were chosen because of their high expression of CXCR4 and low expression of CXCR7 and SDF-1.

[0259] These assays were used to characterize the recombinant humanized versions of anti-CXCR4 antibodies. Variable domains were formatted with human IgG1/k constant domains and cloned into the mammalian expression vector pCEP. Recombinant IgG₁/k-derived antibodies were transiently expressed in HEK293 cells. Expression culture supernatants were filtered and antibodies were purified using protein A sepharose. Purified antibodies were rebuffed in PBS and antibodies concentrations determined by ELISA.

[0260] Recombinants antibody fragments were generated by PCR using oligonucleotides specific for the variable domains of the humanized antibody and subcloning these into an *E. coli* system. Purification of antibody fragments was done by immobilized metal ion affinity chromatography (IMAC).

- Humanization of 515H7 variable domains

[0261] The different sequences alignments of the heavy and light chains variable domains are illustrated in Figures 17 and 18.

[0262] In a first series of experiments, the anti-CXCR4 binding activities of the three first humanized variants were analysed. The VH variant 1 (VH1) was combined with the murine VL and these constructs were evaluated in their capacity to inhibit the binding of a biotinylated murine 515H7 parental antibody. The amino acid sequence of the variable domain of VH1 comprises the SEQ ID No. 90 whereas the nucleotide sequence comprises the SEQ ID No. 91. The amino acid sequence of the full length VH1 comprises the SEQ ID No. 92 whereas the nucleotide sequence comprises the SEQ ID No. 93. This construct showed similar capacity to compete with the murine antibody as the chimeric antibody (Figure 19A). This indicates that the most human VH variant has the same binding capacity as chimeric. Therefore, VH1 was combined with the variant 2 of VL (Figure 19B).

[0263] In further experiments, it has been determined whether humanized variants of the antibody 515H7 inhibit the binding of SDF-1 to CXCR4 expressing cells (Figure 20). The inhibitory capacity of humanized variants of hz515H7 was evaluated by detecting biotinylated SDF-1 in flow cytometry. The humanized antibody hz515H7 VH1 D76N VL2 has a similar capacity to inhibit SDF-1 binding as the chimeric c515H7.

[0264] An antibody fragment of the humanized variant hz515H7 VH1 VL2 has also been tested and it has been determined that said antibody fragment is able to completely inhibit the binding of SDF-1 (Figure 20).

Example 15: Characterization by FACS analysis of anti-CXCR4 humanized Mabs 515H7 binding specificity

[0265] In this experiment, specific binding to human CXCR4 of anti-CXCR4 humanized Mabs 515H7 was examined by FACS analysis.

[0266] NIH3T3, NIH3T3-hCXCR4 transfected were incubated with 0 to 10 μ g/mL of humanized Mabs 515H7 (hz515H7 VH1 D76N VL2, hz515H7 VH1 D76N VL2.1, hz515H7 VH1 D76N VL2.2, hz515H7 VH1 D76N VL2.3) for 20 min at 4°C in the dark in 100 μ l FACS buffer. After 3 washing in FACS buffer, cells were incubated with the secondary antibody, a goat anti-human Alexa 488 (dilution 1/500), for 20 minutes at 4°C in the dark. After 3 washing in FACS buffer, propidium iodide was added in each well and only viable cells were analyzed by FACS. At least 5000 viable cells were assessed to evaluate the mean value of fluorescence intensity for each condition.

[0267] Results of these binding studies are provided in Figure 21 which shows [Mean Fluorescence Intensity (MFI) obtained by FACS] that anti-CXCR4 humanized Mabs hz515H7 bound specifically to human CXCR4-NIH3T3 transfected cell line (MFI= 2.2 with NIH3T3 parent cells)

Example 16: Effect of hz515H7 Mabs on CXCR4 homodimer, by bioluminescence resonance energy transfer (BRET) approach

[0268] This functional assay allows to evaluate the conformational changes induced upon SDF-1 and/or hz515H7 VH1 D76N VL2, hz515H7 VH1 D76N VL2.1, hz515H7 VH1 D76N VL2.2, hz515H7 VH1 D76N VL2.3 binding to CXCR4 receptor at the level of CXCR4 homodimer.

[0269] Expression vectors for 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 homodimerization. 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 CO₂ 5 % in order to allow cell attachment to the plate. Cells were then starved with 200 μ l 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 μ M with or without SDF-1 100 nM in a final volume of 50 μ l. 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).

[0270] Calculation of BRET ratio was performed as previously described (Angers et al., 2000): $[(\text{emission}_{530 \text{ nm}}) - (\text{emission}_{485 \text{ nm}}) \times Cf] / (\text{emission}_{485 \text{ nm}})$, where $Cf = (\text{emission}_{530 \text{ nm}}) / (\text{emission}_{485 \text{ nm}})$ 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.

[0271] SDF1 (100 nM) increased by about 12 % 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 22).

[0272] 515H7 humanized Mabs were able to modulate SDF-1-induced conformational changes for CXCR4 homo-dimers with a percentage of inhibition of SDF-1-induced BRET increase of about 88% for hz515H7 VH1D76N-VL2 Mab, 65% for hz515H7 VH1D76N-VL2.1 Mab, 33% for hz515H7 VH1D76N-VL2.2 Mab and 21% for hz515H7 VH1D76N-VL2.3 Mab (Figure 22)

SEQUENCE LISTING

[0273]

<110> PIERRE FABRE MEDICAMENT KLINGUER-HAMOUR, Christine

EP 2 424 897 B1

<120> NOVEL ANTIBODIES FOR THE TREATMENT OF HIV

<130> 356254 D27470

5 <150> EP09159076.0
<151> 2009-04-29

<150> US61/173680
<151> 2009-04-29

10 <160> 93

<170> PatentIn version 3.5

15 <210> 1
<211> 12
<212> PRT
<213> Mus musculus

20 <400> 1

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

25 <210> 2
<211> 3
<212> PRT
<213> Mus musculus

30 <400> 2

Trp Ala Ser
1

35 <210> 3
<211> 8
<212> PRT
<213> Mus musculus

40 <400> 3

Met Gln Ser Phe Asn Leu Arg Thr
1 5

45 <210> 4
<211> 8
<212> PRT
<213> Mus musculus

50 <400> 4

Gly Phe Thr Phe Thr Asp Asn Tyr
1 5

55 <210> 5
<211> 10
<212> PRT
<213> Mus musculus

<400> 5

Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr
 1 5 10

<210> 6

<211> 11

<212> PRT

<213> Mus musculus

<400> 6

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

<210> 7

<211> 112

<212> PRT

<213> Mus musculus

<400> 7

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> 8

<211> 120

<212> PRT

<213> Mus musculus

<400> 8

EP 2 424 897 B1

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> 9
<211> 17
<212> PRT
<213> Mus musculus

<400> 9

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

Ala

<210> 10
<211> 7
<212> PRT
<213> Mus musculus

<400> 10

Trp Ala Ser Ala Arg Asp Ser
1 5

<210> 11
<211> 5
<212> PRT
<213> Mus musculus

EP 2 424 897 B1

<400> 11

Asp Asn Tyr Met Ser
1 5

5

<210> 12
<211> 19
<212> PRT
<213> Mus musculus

10

<400> 12

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

15

Val Arg Gly

<210> 13
<211> 9
<212> PRT
<213> Mus musculus

20

<400> 13

25

Asp Val Gly Ser Asn Tyr Phe Asp Tyr
1 5

<210> 14
<211> 36
<212> DNA
<213> Mus musculus

30

<400> 14
cagagtctgt tcaacagtcg aaccgaaag aactac 36

35

<210> 15
<211> 9
<212> DNA
<213> Mus musculus

40

<400> 15
tgggcatcc 9

45

<210> 16
<211> 24
<212> DNA
<213> Mus musculus

50

<400> 16
atgcaatctt ttaatcttcg gacg 24

55

<210> 17
<211> 24
<212> DNA
<213> Mus musculus

<400> 17

EP 2 424 897 B1

gggttcacct tcactgataa ctac 24

<210> 18
<211> 30
5 <212> DNA
<213> Mus musculus

<400> 18
10 attagaaaca aagctaattg ttacacaaca 30

<210> 19
<211> 33
<212> DNA
15 <213> Mus musculus

<400> 19
gcaagagatg tcggttccaa ctacttgac tac 33

<210> 20
20 <211> 336
<212> DNA
<213> Mus musculus

<400> 20
25

gacattgtga tgtcacagtc tccatcctcc ctggctgtgt cagcaggaga gaaggtcact 60

atgagctgca aatccagtcga gagtctgttc aacagtcgaa cccgaaagaa ctacttggtc 120

30 **tggtaccagc agaagccagg gcagtcctct aaactgctga tctactgggc atccgctagg 180**

gattctggtg tccctgctcg cttcacaggc agtggatctg agacatattt cactctcacc 240

atcagccgtg tgcaggctga agacctggca gtttattact gcatgcaatc ttttaatctt 300

35 **cggacgttcg gtggaggcac caagctggaa atcaaa 336**

<210> 21
40 <211> 360
<212> DNA
<213> Mus musculus

<400> 21

45 **gaggtgaacc tgggtggagtc tggaggaggc ttggtacagc ctggggggttc tctgagactc 60**

tcctgtgcaa cttctgggtt cacttccact gataactaca tgagttgggt ccgccagcct 120

50 **ccaggaaagg cacttgagtg gttggggcttt attagaaaca aagctaattg ttacacaaca 180**

gactacagtg catctgtgag gggctgggttc accatctcaa gagataattc ccaaagcatc 240

ctctatcttc aaatgaacgc cctgagagcc gaagacagtg ccacttatta ctgtgcaaga 300

55 **gatgtcgggtt ccaactactt tgactactgg ggccaaggca ccactctcac agtctcctca 360**

<210> 22
<211> 51

EP 2 424 897 B1

<212> DNA

<213> Mus musculus

<400> 22

5 aaatccagtc agagtctgtt caacagtcga acccgaaaga actactggc t 51

<210> 23

<211> 21

<212> DNA

10 <213> Mus musculus

<400> 23

tgggcatccg ctagggattc t 21

15 <210> 24

<211> 15

<212> DNA

<213> Mus musculus

20 <400> 24

gataactaca tgagt 15

<210> 25

<211> 57

25 <212> DNA

<213> Mus musculus

<400> 25

30 ttattagaa acaaagctaa tggttacaca acagactaca gtgcatctgt gaggggt 57

<210> 26

<211> 27

<212> DNA

<213> Mus musculus

35 <400> 26

gatgtcgggt ccaactactt tgactac 27

<210> 27

40 <211> 352

<212> PRT

<213> Homo sapiens

<400> 27

45

50

55

EP 2 424 897 B1

	Met	Glu	Gly	Ile	Ser	Ile	Tyr	Thr	Ser	Asp	Asn	Tyr	Thr	Glu	Glu	Met
	1				5					10					15	
5	Gly	Ser	Gly	Asp	Tyr	Asp	Ser	Met	Lys	Glu	Pro	Cys	Phe	Arg	Glu	Glu
				20					25					30		
10	Asn	Ala	Asn	Phe	Asn	Lys	Ile	Phe	Leu	Pro	Thr	Ile	Tyr	Ser	Ile	Ile
			35					40					45			
15	Phe	Leu	Thr	Gly	Ile	Val	Gly	Asn	Gly	Leu	Val	Ile	Leu	Val	Met	Gly
	50						55					60				
20	Tyr	Gln	Lys	Lys	Leu	Arg	Ser	Met	Thr	Asp	Lys	Tyr	Arg	Leu	His	Leu
	65					70					75					80
25	Ser	Val	Ala	Asp	Leu	Leu	Phe	Val	Ile	Thr	Leu	Pro	Phe	Trp	Ala	Val
					85					90					95	
30																
35																
40																
45																
50																
55																

EP 2 424 897 B1

	Asp	Ala	Val	Ala	Asn	Trp	Tyr	Phe	Gly	Asn	Phe	Leu	Cys	Lys	Ala	Val	
				100					105					110			
5	His	Val	Ile	Tyr	Thr	Val	Asn	Leu	Tyr	Ser	Ser	Val	Leu	Ile	Leu	Ala	
			115					120					125				
	Phe	Ile	Ser	Leu	Asp	Arg	Tyr	Leu	Ala	Ile	Val	His	Ala	Thr	Asn	Ser	
10		130					135					140					
	Gln	Arg	Pro	Arg	Lys	Leu	Leu	Ala	Glu	Lys	Val	Val	Tyr	Val	Gly	Val	
	145					150					155					160	
15	Trp	Ile	Pro	Ala	Leu	Leu	Leu	Thr	Ile	Pro	Asp	Phe	Ile	Phe	Ala	Asn	
					165					170					175		
	Val	Ser	Glu	Ala	Asp	Asp	Arg	Tyr	Ile	Cys	Asp	Arg	Phe	Tyr	Pro	Asn	
20				180					185					190			
	Asp	Leu	Trp	Val	Val	Val	Phe	Gln	Phe	Gln	His	Ile	Met	Val	Gly	Leu	
25			195					200					205				
	Ile	Leu	Pro	Gly	Ile	Val	Ile	Leu	Ser	Cys	Tyr	Cys	Ile	Ile	Ile	Ser	
		210					215					220					
30	Lys	Leu	Ser	His	Ser	Lys	Gly	His	Gln	Lys	Arg	Lys	Ala	Leu	Lys	Thr	
	225					230					235					240	
	Thr	Val	Ile	Leu	Ile	Leu	Ala	Phe	Phe	Ala	Cys	Trp	Leu	Pro	Tyr	Tyr	
35				245						250					255		
	Ile	Gly	Ile	Ser	Ile	Asp	Ser	Phe	Ile	Leu	Leu	Glu	Ile	Ile	Lys	Gln	
			260						265					270			
40	Gly	Cys	Glu	Phe	Glu	Asn	Thr	Val	His	Lys	Trp	Ile	Ser	Ile	Thr	Glu	
			275				280						285				
	Ala	Leu	Ala	Phe	Phe	His	Cys	Cys	Leu	Asn	Pro	Ile	Leu	Tyr	Ala	Phe	
45		290					295					300					
	Leu	Gly	Ala	Lys	Phe	Lys	Thr	Ser	Ala	Gln	His	Ala	Leu	Thr	Ser	Val	
50	305					310					315					320	
	Ser	Arg	Gly	Ser	Ser	Leu	Lys	Ile	Leu	Ser	Lys	Gly	Lys	Arg	Gly	Gly	
				325						330					335		
55	His	Ser	Ser	Val	Ser	Thr	Glu	Ser	Glu	Ser	Ser	Ser	Phe	His	Ser	Ser	
				340					345					350			

<210> 28
<211> 356
<212> PRT
<213> Homo sapiens

5

<400> 28

10

15

20

25

30

35

40

45

50

55

EP 2 424 897 B1

	Met	Ser	Ile	Pro	Leu	Pro	Leu	Leu	Gln	Ile	Tyr	Thr	Ser	Asp	Asn	Tyr	
	1				5					10					15		
5	Thr	Glu	Glu	Met	Gly	Ser	Gly	Asp	Tyr	Asp	Ser	Met	Lys	Glu	Pro	Cys	
				20					25					30			
10	Phe	Arg	Glu	Glu	Asn	Ala	Asn	Phe	Asn	Lys	Ile	Phe	Leu	Pro	Thr	Ile	
			35					40					45				
15	Tyr	Ser	Ile	Ile	Phe	Leu	Thr	Gly	Ile	Val	Gly	Asn	Gly	Leu	Val	Ile	
	50						55					60					
20	Leu	Val	Met	Gly	Tyr	Gln	Lys	Lys	Leu	Arg	Ser	Met	Thr	Asp	Lys	Tyr	
	65					70				75						80	
25	Arg	Leu	His	Leu	Ser	Val	Ala	Asp	Leu	Leu	Phe	Val	Ile	Thr	Leu	Pro	
					85					90					95		
30	Phe	Trp	Ala	Val	Asp	Ala	Val	Ala	Asn	Trp	Tyr	Phe	Gly	Asn	Phe	Leu	
				100					105					110			
35	Cys	Lys	Ala	Val	His	Val	Ile	Tyr	Thr	Val	Asn	Leu	Tyr	Ser	Ser	Val	
			115					120					125				
40	Leu	Ile	Leu	Ala	Phe	Ile	Ser	Leu	Asp	Arg	Tyr	Leu	Ala	Ile	Val	His	
	130						135					140					
45	Ala	Thr	Asn	Ser	Gln	Arg	Pro	Arg	Lys	Leu	Leu	Ala	Glu	Lys	Val	Val	
	145					150					155					160	
50	Tyr	Val	Gly	Val	Trp	Ile	Pro	Ala	Leu	Leu	Leu	Thr	Ile	Pro	Asp	Phe	
					165					170					175		
55	Ile	Phe	Ala	Asn	Val	Ser	Glu	Ala	Asp	Asp	Arg	Tyr	Ile	Cys	Asp	Arg	
				180					185					190			
60	Phe	Tyr	Pro	Asn	Asp	Leu	Trp	Val	Val	Val	Phe	Gln	Phe	Gln	His	Ile	
			195					200					205				
65	Met	Val	Gly	Leu	Ile	Leu	Pro	Gly	Ile	Val	Ile	Leu	Ser	Cys	Tyr	Cys	
	210						215					220					
70	Ile	Ile	Ile	Ser	Lys	Leu	Ser	His	Ser	Lys	Gly	His	Gln	Lys	Arg	Lys	
	225					230					235					240	

EP 2 424 897 B1

	Ala	Leu	Lys	Thr	Thr	Val	Ile	Leu	Ile	Leu	Ala	Phe	Phe	Ala	Cys	Trp	
					245					250					255		
5	Leu	Pro	Tyr	Tyr	Ile	Gly	Ile	Ser	Ile	Asp	Ser	Phe	Ile	Leu	Leu	Glu	
				260					265					270			
10	Ile	Ile	Lys	Gln	Gly	Cys	Glu	Phe	Glu	Asn	Thr	Val	His	Lys	Trp	Ile	
			275					280					285				
15	Ser	Ile	Thr	Glu	Ala	Leu	Ala	Phe	Phe	His	Cys	Cys	Leu	Asn	Pro	Ile	
	290						295					300					
20	Leu	Tyr	Ala	Phe	Leu	Gly	Ala	Lys	Phe	Lys	Thr	Ser	Ala	Gln	His	Ala	
	305					310					315					320	
25	Leu	Thr	Ser	Val	Ser	Arg	Gly	Ser	Ser	Leu	Lys	Ile	Leu	Ser	Lys	Gly	
					325					330					335		
30	Lys	Arg	Gly	Gly	His	Ser	Ser	Val	Ser	Thr	Glu	Ser	Glu	Ser	Ser	Ser	
			340						345					350			
35	Phe	His	Ser	Ser													
			355														
40																	
45																	
50																	
55																	

<210> 29
 <211> 360
 <212> PRT
 <213> Homo sapiens
 <400> 29

EP 2 424 897 B1

1	Met	Glu	Asp	Phe	Asn	Met	Glu	Ser	Asp	Ser	Phe	Glu	Asp	Phe	Trp	Lys
					5					10					15	
5	Gly	Glu	Asp	Leu	Ser	Asn	Tyr	Ser	Tyr	Ser	Ser	Thr	Leu	Pro	Pro	Phe
				20					25					30		
10	Leu	Leu	Asp	Ala	Ala	Pro	Cys	Glu	Pro	Glu	Ser	Leu	Glu	Ile	Asn	Lys
			35					40					45			
15	Tyr	Phe	Val	Val	Ile	Ile	Tyr	Ala	Leu	Val	Phe	Leu	Leu	Ser	Leu	Leu
		50					55							60		
20	Gly	Asn	Ser	Leu	Val	Met	Leu	Val	Ile	Leu	Tyr	Ser	Arg	Val	Gly	Arg
	65					70					75					80
25	Ser	Val	Thr	Asp	Val	Tyr	Leu	Leu	Asn	Leu	Ala	Leu	Ala	Asp	Leu	Leu
					85					90					95	
30	Phe	Ala	Leu	Thr	Leu	Pro	Ile	Trp	Ala	Ala	Ser	Lys	Val	Asn	Gly	Trp
35																
40																
45																
50																
55																

EP 2 424 897 B1

	100		105		110
5	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
10	Tyr Leu Ala Ile Val His Ala Thr Arg Thr Leu Thr Gln Lys Arg Tyr	145	150		155
	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
20	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
30	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
40	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
	Phe Arg His Gly Leu Leu Lys Ile Leu Ala Ile His Gly Leu Ile Ser	325	330		335
50	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		

EP 2 424 897 B1

<210> 30
 <211> 8
 <212> PRT
 <213> Mus musculus
 5
 <400> 30

 Lys Gln Ser Tyr Asn Leu Arg Thr
 1 5
 10

 <210> 31
 <211> 8
 <212> PRT
 <213> Mus musculus
 15
 <400> 31

 Gly Phe Ser Leu Thr Asp Tyr Gly
 1 5
 20

 <210> 32
 <211> 7
 <212> PRT
 <213> Mus musculus
 25
 <400> 32

 Ile Trp Gly Asp Gly Thr Thr
 1 5
 30

 <210> 33
 <211> 10
 <212> PRT
 <213> Mus musculus
 35
 <400> 33

 Ala Arg Gly Arg Gln Phe Gly Phe Asp Tyr
 1 5 10
 40

 <210> 34
 <211> 112
 <212> PRT
 <213> Mus musculus
 45
 <400> 34
 50

 55

EP 2 424 897 B1

	Asp	Ile	Val	Met	Ser	Gln	Ser	Pro	Ser	Ser	Leu	Ala	Val	Ser	Ala	Gly
	1				5					10					15	
5	Glu	Lys	Val	Thr	Met	Arg	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
10			35					40					45			
	Ser	Pro	Lys	Leu	Leu	Ile	Phe	Trp	Ala	Ser	Ile	Arg	Glu	Ser	Gly	Val
		50					55					60				
15	Pro	Asp	Arg	Phe	Thr	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr
	65					70					75					80
	Ile	Ser	Ser	Val	Gln	Ala	Glu	Asp	Leu	Ala	Val	Tyr	Tyr	Cys	Lys	Gln
20					85					90					95	
	Ser	Tyr	Asn	Leu	Arg	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys
25				100					105					110		

<210> 35

<211> 116

<212> PRT

30 <213> Mus musculus

<400> 35

35

40

45

50

55

EP 2 424 897 B1

1 Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln
 5 Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Asp Tyr
 10 Gly Val Tyr Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
 15 Gly Met Ile Trp Gly Asp Gly Thr Thr Asp Tyr Asn Ser Ala Leu Lys
 20 Ser Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Phe Leu
 25 Lys Met Asn Thr Leu Gln Thr Asp Asp Thr Ala Arg Tyr Tyr Cys Ala
 30 Arg Gly Arg Gln Phe Gly Phe Asp Tyr Trp Gly Gln Gly Thr Thr Leu
 35 Thr Val Ser Ser
 40 Trp Ala Ser Ile Arg Glu Ser
 45 Lys Gln Ser Tyr Asn Leu Arg Thr
 50
 55
 <210> 36
 <211> 7
 <212> PRT
 <213> Mus musculus
 <400> 36
 <210> 37
 <211> 8
 <212> PRT
 <213> Mus musculus
 <400> 37
 <210> 38
 <211> 5
 <212> PRT
 <213> Mus musculus
 <400> 38

EP 2 424 897 B1

Asp Tyr Gly Val Tyr
1 5

5 <210> 39
<211> 16
<212> PRT
<213> Mus musculus

10 <400> 39

Met Ile Trp Gly Asp Gly Thr Thr Asp Tyr Asn Ser Ala Leu Lys Ser
1 5 10 15

15 <210> 40
<211> 8
<212> PRT
<213> Mus musculus

20 <400> 40

Gly Arg Gln Phe Gly Phe Asp Tyr
1 5

25 <210> 41
<211> 36
<212> DNA
<213> Mus musculus

30 <400> 41
cagagtctgt tcaacagtag aaccgaaag aactac 36

<210> 42
<211> 24
35 <212> DNA
<213> Mus musculus

<400> 42
aagcaatctt ataatcttcg gacg 24

40 <210> 43
<211> 24
<212> DNA
<213> Mus musculus

45 <400> 43
gggttctcat taaccgacta tggt 24

<210> 44
50 <211> 21
<212> DNA
<213> Mus musculus

<400> 44
55 atatggggtg atggaaccac a 21

<210> 45
<211> 30

EP 2 424 897 B1

<212> DNA

<213> Mus musculus

<400> 45

5 gccaggggta gacagtggg gtttgactac 30

<210> 46

<211> 336

<212> DNA

10 <213> Mus musculus

<400> 46

15 gacattgtga tgtcacagtc tccatcctcc ctggctgtgt cagcaggaga gaaggctact 60

atgagggtgca aatccagtc gagtctgttc aacagtagaa cccgaaagaa ctacttggct 120

tggtagcaaac agaaaccagg gcagtctcct aaactgctga tcttctgggc atccattagg 180

20 gaatctgggg tccctgatcg cttcacaggc agtggatctg ggacagattt cactctcacc 240

atcagcagtg tgcaggctga agacctggca gtttattact gcaagcaatc ttataatctt 300

cggacgttcg gtggaggcac caagctggaa atcaaa 336

25

<210> 47

<211> 348

<212> DNA

<213> Mus musculus

30

<400> 47

caggtgcagc tgaaggagtc tgggcctggc ctggtggcgc cctcacagag cctgtccatc 60

35 acatgcaccg tctcagggtt ctcattaacc gactatggtg tatactgggt tcgccagcct 120

ccaggaaagg gtctggagtg gctgggaatg atatggggtg atggaaccac agactataat 180

40 tcagctctca aatccagact gagcatcagt aaggacaact ccaagagcca agttttctta 240

aaaatgaaca ctctgcaaac tgatgacaca gccaggtatt actgtgccag gggtagacag 300

ttcgggtttg actactgggg ccaaggcacc acgctcacag tctcctca 348

45

<210> 48

<211> 51

<212> DNA

<213> Mus musculus

50

<400> 48

aaatccagtc agagtctgtt caacagtaga acccgaaaga actactggc t 51

<210> 49

<211> 21

55

<212> DNA

<213> Mus musculus

<400> 49

EP 2 424 897 B1

tgggcatcca ttagggaatc t 21

<210> 50

<211> 24

<212> DNA

<213> Mus musculus

<400> 50

aagcaatctt ataatcttcg gacg 24

<210> 51

<211> 15

<212> DNA

<213> Mus musculus

<400> 51

gactatggtg tatac 15

<210> 52

<211> 48

<212> DNA

<213> Mus musculus

<400> 52

atgatatggg gtgatggaac cacagactat aattcagctc tcaaatcc 48

<210> 53

<211> 24

<212> DNA

<213> Mus musculus

<400> 53

ggtagacagt tcgggttga ctac 24

<210> 54

<211> 359

<212> PRT

<213> Mus musculus

<400> 54

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

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

EP 2 424 897 B1

	Tyr	Met	Ser	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val
			35					40					45			
5	Gly	Phe	Ile	Arg	Asn	Lys	Ala	Asn	Gly	Tyr	Thr	Thr	Glu	Tyr	Ala	Ala
	50						55					60				
10	Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asp	Ser	Lys	Ser	Ile
	65					70					75					80
	Ala	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Lys	Thr	Glu	Asp	Thr	Ala	Val	Tyr
					85					90					95	
15	Tyr	Cys	Ala	Arg	Asp	Val	Gly	Ser	Asn	Tyr	Phe	Asp	Tyr	Trp	Gly	Gln
				100					105					110		
20	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Gln	Leu	Lys	Ser	Ser	Gly	Ser	Gly
			115						120					125		
25	Ser	Glu	Ser	Lys	Ser	Thr	Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Ser	Ser
		130					135					140				
	Leu	Ala	Val	Ser	Leu	Gly	Glu	Arg	Ala	Thr	Met	Ser	Cys	Lys	Ser	Ser
	145					150					155					160
30	Gln	Ser	Leu	Phe	Asn	Ser	Arg	Thr	Arg	Lys	Asn	Tyr	Leu	Ala	Trp	Tyr
					165					170					175	
35	Gln	Gln	Lys	Pro	Gly	Gln	Ser	Pro	Lys	Leu	Leu	Ile	Tyr	Trp	Ala	Ser
				180					185					190		
	Ala	Arg	Asp	Ser	Gly	Val	Pro	Ala	Arg	Phe	Thr	Gly	Ser	Gly	Ser	Glu
			195					200					205			
40	Thr	Tyr	Phe	Thr	Leu	Thr	Ile	Ser	Arg	Val	Gln	Ala	Glu	Asp	Leu	Ala
		210					215					220				
45	Val	Tyr	Tyr	Cys	Met	Gln	Ser	Phe	Asn	Leu	Arg	Thr	Phe	Gly	Gln	Gly
	225					230					235					240
50	Thr	Lys	Val	Glu	Ile	Lys	Thr	Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe
					245					250					255	
	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val
				260					265					270		
55	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp
			275					280					285			

EP 2 424 897 B1

Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr
 290 295 300

5 Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr
 305 310 315 320

10 Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val
 325 330 335

15 Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly
 340 345 350

Glu His His His His His His
 355

20 <210> 55
 <211> 1077
 <212> DNA
 <213> Mus musculus

25 <400> 55

30

35

40

45

50

55

EP 2 424 897 B1

	gaggtgcagc tgggtggagtc tggcggagga ctggtgcagc ccggcagaag cctgagactg	60
	agctgcaccg ccagcggctt caccttcacc gacaactaca tgtcctgggt gcgccaggcc	120
5	cctggaaagg gcctggaatg ggtgggcttc atccggaaca aggccaacgg ctacaccaca	180
	gagtacgccg ccagcgtgaa gggccggctc accatcagcc gggacgacag caagagcatt	240
	gcctacctgc agatgaacag cctgaaaacc gaggacaccg ccgtgtacta ctgcgccagg	300
10	gacgtgggca gcaactactt cgactactgg ggccagggca cactggtgac cgtgtctagc	360
	caattgaaaa gcagcggcag cggtagcgaa agcaagtcga ccgacatcgt gatgaccag	420
	agccccagca gcctggccgt gtctctgggc gagcgggcca ccatgagctg caagagcagc	480
15	cagagcctgt tcaacagccg gacccggaag aactacctgg cctggatatca gcagaagccc	540
	ggccagtccc ccaagctgct gatctactgg gccagcgcca gagatagcgg cgtgcccgct	600
20	cgctttaccg gcagcggcag cgagacctac ttcacctga ccatcagccg ggtgcaggcc	660
	gaggacctcg ccgtgtacta ctgcatgcag agcttcaacc tgccggacctt cggccagggc	720
	accaaggtgg agatcaagac gcgtacggtg gccgctccca gcgtgttcat cttcccccca	780
25	agcgacgagc agctgaagag cggcacccgc agcgtggtgt gtctgctgaa caacttctac	840
	cccagggagg ccaaggtgca gtggaaggtg gacaacgccc tgcagagcgg caacagccag	900
30	gagagcgtca ccgagcagga cagcaaggac tccacctaca gcctgagcag caccctgacc	960
	ctgagcaagg ccgactacga gaagcacaag gtgtacgcct gtgaggtgac ccaccagggc	1020
	ctgtccagcc ccgtgaccaa gagcttcaac aggggcgagc accatcatca ccaccat	1077
35	<210> 56	
	<211> 446	
	<212> PRT	
	<213> Mus musculus	
40	<400> 56	
45		
50		
55		

EP 2 424 897 B1

	Glu	Val	Asn	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly	
	1				5					10					15		
5	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Thr	Ser	Gly	Phe	Thr	Phe	Thr	Asp	Asn	
				20					25					30			
10	Tyr	Met	Ser	Trp	Val	Arg	Gln	Pro	Pro	Gly	Lys	Ala	Leu	Glu	Trp	Leu	
			35					40					45				
15	Gly	Phe	Ile	Arg	Asn	Lys	Ala	Asn	Gly	Tyr	Thr	Thr	Asp	Tyr	Ser	Ala	
		50					55					60					
20	Ser	Val	Arg	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ser	Gln	Ser	Ile	
	65					70				75						80	
25	Leu	Tyr	Leu	Gln	Met	Asn	Ala	Leu	Arg	Ala	Glu	Asp	Ser	Ala	Thr	Tyr	
					85				90						95		
30	Tyr	Cys	Ala	Arg	Asp	Val	Gly	Ser	Asn	Tyr	Phe	Asp	Tyr	Trp	Gly	Gln	
				100					105					110			
35	Gly	Thr	Thr	Leu	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	
			115					120					125				
40	Phe	Pro	Leu	Ala	Pro	Cys	Ser	Arg	Ser	Thr	Ser	Glu	Ser	Thr	Ala	Ala	
		130					135					140					
45	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	
	145					150					155					160	
50	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	
					165					170					175		
55	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	
				180					185					190			
60	Ser	Ser	Ser	Leu	Gly	Thr	Lys	Thr	Tyr	Thr	Cys	Asn	Val	Asp	His	Lys	
			195					200					205				
65	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Ser	Lys	Tyr	Gly	Pro	
		210					215					220					
70	Pro	Cys	Pro	Ser	Cys	Pro	Ala	Pro	Glu	Phe	Leu	Gly	Gly	Pro	Ser	Val	
	225					230					235					240	

EP 2 424 897 B1

	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	
					245					250					255		
5	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	Gln	Glu	Asp	Pro	Glu	
				260					265					270			
10	Val	Gln	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	
			275					280					285				
15	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Phe	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	
	290						295					300					
20	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	
	305					310					315					320	
25	Cys	Lys	Val	Ser	Asn	Lys	Gly	Leu	Pro	Ser	Ser	Ile	Glu	Lys	Thr	Ile	
					325					330					335		
30	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	
				340					345					350			
35	Pro	Ser	Gln	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	
			355					360					365				
40	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	
	370						375					380					
45	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	
	385					390					395					400	
50	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Arg	Leu	Thr	Val	Asp	Lys	Ser	Arg	
				405						410					415		
55	Trp	Gln	Glu	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	
				420					425					430			
60	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Leu	Gly			
			435					440					445				
65	<210>	57															
70	<211>	446															
75	<212>	PRT															
80	<213>	Mus musculus															
85	<400>	57															

EP 2 424 897 B1

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

5 Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Phe Thr Phe Thr Asp Asn

10

15

20

25

30

35

40

45

50

55

EP 2 424 897 B1

	20	25	30
5	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		
10	Ser Val Arg Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gln Ser Ile 65 70 75 80		
15	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		
20	Gly Thr Thr Leu Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val 115 120 125		
25	Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala 130 135 140		
	Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser 145 150 155 160		
30	Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val 165 170 175		
35	Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro 180 185 190		
	Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys 195 200 205		
40	Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro 210 215 220		
45	Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val 225 230 235 240		
	Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr 245 250 255		
50	Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu 260 265 270		
55	Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys 275 280 285		

EP 2 424 897 B1

	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Phe	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	
	290						295					300					
5	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	
	305					310					315					320	
10	Cys	Lys	Val	Ser	Asn	Lys	Gly	Leu	Pro	Ser	Ser	Ile	Glu	Lys	Thr	Ile	
					325					330					335		
15	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	
				340					345					350			
20	Pro	Ser	Gln	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	
			355					360					365				
25	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	
	370						375					380					
30	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	
	385					390					395				400		
35	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Arg	Leu	Thr	Val	Asp	Lys	Ser	Arg	
				405						410					415		
40	Trp	Gln	Glu	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	
				420					425					430			
45	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Leu	Gly			
			435					440					445				
50	<210>	58															
	<211>	445															
55	<212>	PRT															
	<213>	Mus musculus															
60	<400>	58															
65	Glu	Val	Asn	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly	
	1				5					10					15		
70	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Thr	Ser	Gly	Phe	Thr	Phe	Thr	Asp	Asn	
			20						25					30			
75	Tyr	Met	Ser	Trp	Val	Arg	Gln	Pro	Pro	Gly	Lys	Ala	Leu	Glu	Trp	Leu	
			35				40						45				
80	Gly	Phe	Ile	Arg	Asn	Lys	Ala	Asn	Gly	Tyr	Thr	Thr	Asp	Tyr	Ser	Ala	
	50						55					60					

EP 2 424 897 B1

	Ser Val Arg Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gln Ser Ile	65	70	75	80
5	Leu Tyr Leu Gln Met Asn Ala Leu Arg Ala Glu Asp Ser Ala Thr Tyr	85	90	95	
10	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 Ala Ser Thr Lys Gly Pro Ser Val	115	120	125	
15	Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala	130	135	140	
20	Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser	145	150	155	160
	Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val	165	170	175	
25	Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro	180	185	190	
30	Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys	195	200	205	
	Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val	210	215	220	
35	Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe	225	230	235	240
40	Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro	245	250	255	
	Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val	260	265	270	
45	Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr	275	280	285	
50	Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val	290	295	300	
	Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys	305	310	315	320
55	Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser				

	325		330		335
5	Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro	340	345	350	
10	Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val	355	360	365	
15	Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly	370	375	380	
20	Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp	385	390	395	400
25	Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp	405	410	415	
30	Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His	420	425	430	
35	Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly	435	440	445	
40	<210> 59				
45	<211> 219				
50	<212> PRT				
55	<213> Mus musculus				
	<400> 59				

EP 2 424 897 B1

	Asp	Ile	Val	Met	Ser	Gln	Ser	Pro	Ser	Ser	Leu	Ala	Val	Ser	Ala	Gly	
	1				5					10					15		
5	Glu	Lys	Val	Thr	Met	Ser	Cys	Lys	Ser	Ser	Gln	Ser	Leu	Phe	Asn	Ser	
				20					25					30			
10	Arg	Thr	Arg	Lys	Asn	Tyr	Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	
				35				40					45				
15	Ser	Pro	Lys	Leu	Leu	Ile	Tyr	Trp	Ala	Ser	Ala	Arg	Asp	Ser	Gly	Val	
		50					55					60					
20	Pro	Ala	Arg	Phe	Thr	Gly	Ser	Gly	Ser	Glu	Thr	Tyr	Phe	Thr	Leu	Thr	
	65					70					75					80	
25	Ile	Ser	Arg	Val	Gln	Ala	Glu	Asp	Leu	Ala	Val	Tyr	Tyr	Cys	Met	Gln	
					85					90					95		
30	Ser	Phe	Asn	Leu	Arg	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys	
				100					105					110			
35	Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	
				115				120					125				
40	Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	
		130					135					140					
45	Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	
	145					150					155					160	
50	Ser	Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	
					165					170					175		
55	Thr	Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	
				180					185					190			
60	Lys	His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	
			195					200					205				
65	Pro	Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys						
		210					215										

<210> 60

<211> 1341

<212> DNA

<213> Mus musculus

<400> 60

	gaagtgaacc tgggtggagtc tggcgggcgga ctggtgcagc ctggggggcag cctgagactg	60
	agctgcgcca cctccggctt caccttcacc gacaactaca tgagctgggt gcgccagccc	120
5	cctggcaagg ccctggaatg gctgggcttc atccggaaca aggccaacgg ctacaccacc	180
	gactacagcg ccagcgtgcg gggcagattc accatcagcc gggacaacag ccagagcatc	240
	ctgtacctgc agatgaacgc cctgcggggc gaggacagcg ccacctacta ctgtgcccg	300
10	gacgtgggca gcaactactt cgactactgg ggccagggca ccacactgac cgtgtccagc	360
	gccagcacca agggcccaag cgtgttcccc ctggccccct gctccagaag caccagcgag	420
15	agcacagccg ccctgggctg cctggtgaag gactacttcc ccgagcccgt gaccgtgtcc	480
	tggaaacagcg gagccctgac cagcggcgtg cacaccttcc ccgccgtgct gcagagcagc	540
	ggcctgtaca gcctgagcag cgtggtgacc gtgccagca gcagcctggg caccaagacc	600
20	tacacctgta acgtggacca caagcccagc aacaccaagg tggacaagag ggtggagagc	660
	aagtacggcc caccctgccc cagctgccc gcccccgagt tcctgggcgg acccagcgtg	720
25	ttcctgttcc cccccaagcc caaggacacc ctgatgatca gcagaacccc cgaggtgacc	780
	tgtgtggtgg tggacgtgtc ccaggaggac ccgaggtcc agttcaactg gtacgtggac	840
	ggcgtggagg tgcacaacgc caagaccaag ccagagagg agcagtttaa cagcacctac	900
30	cgggtggtgt ccgtgctgac cgtgctgcac caggactggc tgaacggcaa agagtacaag	960
	tgtaaagtct ccaacaaggg cctgccaaag agcatcgaaa agaccatcag caaggccaag	1020
35	ggccagccta gagagcccca ggtctacacc ctgccacca gccaagagga gatgaccaag	1080
	aaccaggtgt ccctgacctg tctggtgaag ggcttctacc caagcgacat cgccgtggag	1140
	tgggagagca acggccagcc cgagaacaac tacaagacca ccccccgagt gctggacagc	1200
40	gacggcagct tcttcctgta cagcaggctg accgtggaca agtccagatg gcaggagggc	1260
	aacgtcttta gctgctccgt gatgcacgag gccctgcaca accactacac ccagaagagc	1320
	ctgagcctgt ccctgggctg a	1341
45	<210> 61	
	<211> 1341	
	<212> DNA	
	<213> Mus musculus	
50	<400> 61	
55		

	gaagtgaacc tgggtggagtc tggcggcgga ctggtgcagc ctgggggag cctgagactg	60
	agctgcgcca cctccggctt caccttcacc gacaactaca tgagctgggt gcgccagccc	120
5	cctggcaagg ccctggaatg gctgggcttc atccggaaca aggccaacgg ctacaccacc	180
	gactacagcg ccagcgtgcg gggcagattc accatcagcc gggacaacag ccagagcatc	240
	ctgtacctgc agatgaacgc cctgcggggc gaggacagcg ccacctacta ctgtgcccgg	300
10	gacgtgggca gcaactactt cgactactgg ggccagggca ccacactgac cgtgtccagc	360
	gccagcacca agggcccaag cgtgttcccc ctggccccct gctccagaag caccagcgag	420
15	agcacagccg ccctgggctg cctggtgaag gactacttcc ccgagcccgt gaccgtgtcc	480
	tgggaacagcg gagccctgac cagcggcgtg cacaccttcc ccgccgtgct gcagagcagc	540
	ggcctgtaca gcctgagcag cgtggtgacc gtgccagca gcagcctggg caccaagacc	600
20	tacacctgta acgtggacca caagcccagc aacaccaagg tggacaagag ggtggagagc	660
	aagtacggcc caccctgccc cccctgccc gcccccgagt tcctgggagg acccagcgtg	720
25	ttcctgttcc cccccaagcc caaggacacc ctgatgatca gcagaacccc cgaggtgacc	780
	tgtgtggtgg tggacgtgtc ccaggaggac ccgaggtcc agttcaactg gtacgtggac	840
	ggcgtggagg tgcacaacgc caagaccaag ccagagagg agcagtttaa cagcacctac	900
30	cgggtggtgt ccgtgctgac cgtgctgcac caggactggc tgaacggcaa agagtacaag	960
	tgtaaggtct ccaacaaggg cctgccaaag agcatcgaaa agaccatcag caaggccaag	1020
	ggccagccta gagagcccca ggtctacacc ctgccacca gccaaagagga gatgaccaag	1080
35	aaccaggtgt ccctgacctg tctggtgaag ggcttctacc caagcgacat cgccgtggag	1140
	tgggagagca acggccagcc cgagaacaac tacaagacca cccccccagt gctggacagc	1200
40	gacggcagct tcttcctgta cagcaggctg accgtggaca agtccagatg gcaggagggc	1260
	aacgtcttta gctgctccgt gatgcacgag gccctgcaca accactacac ccagaagagc	1320
45	ctgagcctgt ccctgggctg a	1341
	<210> 62	
	<211> 1338	
	<212> DNA	
50	<213> Mus musculus	
	<400> 62	
55		

	gaagtgaacc tgggtggagtc tggcggcgga ctggtgcagc ctggggggcag cctgagactg	60
	agctgcgcca cctccggctt caccttcacc gacaactaca tgagctgggt gcgccagccc	120
5	cctggcaagg ccctggaatg gctgggcttc atccggaaca aggccaacgg ctacaccacc	180
	gactacagcg ccagcgtgcg gggcagattc accatcagcc gggacaacag ccagagcatc	240
10	ctgtacctgc agatgaacgc cctgcggggc gaggacagcg ccacctacta ctgtgcccg	300
	gacgtgggca gcaactactt cgactactgg ggccagggca ccacactgac cgtgtccagc	360
	gccagcacca agggcccaag cgtgttcccc ctggccccct gctccagaag caccagcgag	420
15	agcacagccg ccctgggctg cctggtgaag gactacttcc ccgagcccgt gaccgtgtcc	480
	tggaaacagcg gagccctgac cagcggcgtg cacaccttcc ccgccgtgct gcagagcagc	540
	ggcctgtaca gcctgagcag cgtggtgacc gtgccaaagca gcaacttcgg caccagacc	600
20	tacacctgta acgtggacca caagcccagc aacaccaagg tggacaagac cgtggagagg	660
	aagtgtgtg tggagtgcc cccctgccc gccccccag tggccggacc cagcgtgttc	720
25	ctgttcccc ccaagcccaa ggacacctg atgatcagca gaacccccga ggtgacctgt	780
	gtggtggtgg acgtgtccc cgaggacccc gaggtgcagt tcaactggta cgtggacggc	840
	gtggaggtgc acaacgcaa gaccaagccc agagaggagc agtttaacag caccttcgg	900
30	gtggtgtccg tgctgaccgt ggtgcaccag gactggctga acggcaagga gtacaagtgt	960
	aaggtctcca acaagggcct gccagcccc atcgaaaaga ccatcagcaa gaccaaggga	1020
	cagccaagag agccacaggt ctacacctg cccccagca gggaggagat gaccaagaac	1080
35	caggtgtccc tgacctgtct ggtgaagggc ttctacccaa gcgacatcgc cgtggagtgg	1140
	gagagcaacg gccagcccga gaacaactac aagaccaccc ccccaatgct ggacagcgac	1200
40	ggcagcttct tcctgtacag caagctgaca gtggacaaga gcagatggca gcagggaac	1260
	gtgttcagct gctccgtgat gcacgaggcc ctgcacaacc actacacca gaagagcctg	1320
	agcctgtccc caggctga	1338
45	<210> 63 <211> 660 <212> DNA <213> Mus musculus	
50	<400> 63	
	gacatcgtga tgagccagag cccagcagc ctggccgtgt ctgccggcga gaaagtgacc	60
55		

EP 2 424 897 B1

atgagctgca agagcagcca gagcctgttc aacagccgga cccggaagaa ctacctggcc 120
 tggatatcagc agaagcccgg ccagtccccc aagctgctga tctactgggc cagcgccaga 180
 5 gacagcggcg tgcccgccag attcaccggc agcggcagcg agacatactt caccctgacc 240
 atcagccggg tgcaggccga ggatctggcc gtgtactact gcatgcagag cttcaacctg 300
 10 cggacctttg gcggcggaac aaagctggaa atcaagcgta cggtgggccgc tcccagcgtg 360
 ttcattcttc ccccaagcga cgagcagctg aagagcggca ccgccagcgt ggtgtgtctg 420
 ctgaacaact tctaccccag ggaggccaag gtgcagtgga aggtggacaa cgccctgcag 480
 15 agcggcaaca gccaggagag cgtcaccgag caggacagca aggactccac ctacagcctg 540
 agcagcaccc tgaccctgag caaggccgac tacgagaagc acaaggtgta cgcctgtgag 600
 gtgaccaccc agggcctgtc cagccccgtg accaagagct tcaacagggg cgagtgtgta 660

<210> 64
 <211> 120
 <212> PRT
 <213> Mus musculus
 <400> 64

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Thr Asp Asn
 20 25 30
 Tyr Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Gly Phe Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr Glu Tyr Ala Ala
 50 55 60
 Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Ser Ile
 65 70 75 80
 Ala Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val 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 Leu Val Thr Val Ser Ser
 115 120

<210> 65

EP 2 424 897 B1

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

5 <400> 65

10	Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ala	Val	Ser	Leu	Gly
	1				5					10					15	
15	Glu	Arg	Ala	Thr	Met	Ser	Cys	Lys	Ser	Ser	Gln	Ser	Leu	Phe	Asn	Ser
				20					25					30		
20	Arg	Thr	Arg	Lys	Asn	Tyr	Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln
				35				40					45			
25	Ser	Pro	Lys	Leu	Leu	Ile	Tyr	Trp	Ala	Ser	Ala	Arg	Asp	Ser	Gly	Val
		50					55					60				
30	Pro	Ala	Arg	Phe	Thr	Gly	Ser	Gly	Ser	Glu	Thr	Tyr	Phe	Thr	Leu	Thr
	65					70					75					80
35	Ile	Ser	Arg	Val	Gln	Ala	Glu	Asp	Leu	Ala	Val	Tyr	Tyr	Cys	Met	Gln
					85					90					95	
40	Ser	Phe	Asn	Leu	Arg	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys
				100					105					110		

<210> 66
 <211> 112
 <212> PRT
 <213> Mus musculus

40 <400> 66

45

50

55

EP 2 424 897 B1

	Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Asp	Ser	Leu	Ala	Val	Ser	Leu	Gly
	1				5					10					15	
5	Glu	Arg	Ala	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
10				35				40					45			
	Pro	Pro	Lys	Leu	Leu	Ile	Tyr	Trp	Ala	Ser	Ala	Arg	Asp	Ser	Gly	Val
		50					55					60				
15	Pro	Asp	Arg	Phe	Ser	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
20					85					90					95	
	Ser	Phe	Asn	Leu	Arg	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys
25				100					105					110		

<210> 67

<211> 446

<212> PRT

30 <213> Mus musculus

<400> 67

35

40

45

50

55

EP 2 424 897 B1

	Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Arg	
	1				5					10					15		
5	Ser	Leu	Arg	Leu	Ser	Cys	Thr	Ala	Ser	Gly	Phe	Thr	Phe	Thr	Asp	Asn	
				20					25					30			
10	Tyr	Met	Ser	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val	
			35					40					45				
15	Gly	Phe	Ile	Arg	Asn	Lys	Ala	Asn	Gly	Tyr	Thr	Thr	Glu	Tyr	Ala	Ala	
		50					55					60					
20	Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ser	Lys	Ser	Ile	
	65					70					75					80	
25	Ala	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Lys	Thr	Glu	Asp	Thr	Ala	Val	Tyr	
					85					90					95		
30	Tyr	Cys	Ala	Arg	Asp	Val	Gly	Ser	Asn	Tyr	Phe	Asp	Tyr	Trp	Gly	Gln	
				100					105					110			
35	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	
			115					120					125				
40	Phe	Pro	Leu	Ala	Pro	Cys	Ser	Arg	Ser	Thr	Ser	Glu	Ser	Thr	Ala	Ala	
		130					135					140					
45	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	
	145					150					155					160	
50	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	
					165					170					175		
55	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	
				180					185					190			
60	Ser	Ser	Ser	Leu	Gly	Thr	Lys	Thr	Tyr	Thr	Cys	Asn	Val	Asp	His	Lys	
			195					200					205				
65	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Ser	Lys	Tyr	Gly	Pro	
		210					215					220					
70	Pro	Cys	Pro	Ser	Cys	Pro	Ala	Pro	Glu	Phe	Leu	Gly	Gly	Pro	Ser	Val	

EP 2 424 897 B1

	225		230		235		240									
5	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr
				245					250						255	
10	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	Gln	Glu	Asp	Pro	Glu
				260					265					270		
15	Val	Gln	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys
			275					280					285			
20	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Phe	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser
		290					295					300				
25	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys
	305					310					315					320
30	Cys	Lys	Val	Ser	Asn	Lys	Gly	Leu	Pro	Ser	Ser	Ile	Glu	Lys	Thr	Ile
					325					330					335	
35	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro
				340					345					350		
40	Pro	Ser	Gln	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu
			355					360					365			
45	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn
	370						375					380				
50	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser
	385					390					395					400
55	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Arg	Leu	Thr	Val	Asp	Lys	Ser	Arg
				405						410					415	
60	Trp	Gln	Glu	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu
				420					425					430		
65	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Leu	Gly		
			435					440					445			

<210> 68

<211> 446

<212> PRT

<213> Mus musculus

<400> 68

EP 2 424 897 B1

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

5

10

15

20

25

30

35

40

45

50

55

EP 2 424 897 B1

	Ser	Leu	Arg	Leu	Ser	Cys	Thr	Ala	Ser	Gly	Phe	Thr	Phe	Thr	Asp	Asn	
				20					25					30			
5	Tyr	Met	Ser	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val	
			35					40					45				
10	Gly	Phe	Ile	Arg	Asn	Lys	Ala	Asn	Gly	Tyr	Thr	Thr	Glu	Tyr	Ala	Ala	
		50					55					60					
15	Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ser	Lys	Ser	Ile	
	65					70				75						80	
20	Ala	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Lys	Thr	Glu	Asp	Thr	Ala	Val	Tyr	
				85					90						95		
25	Tyr	Cys	Ala	Arg	Asp	Val	Gly	Ser	Asn	Tyr	Phe	Asp	Tyr	Trp	Gly	Gln	
				100					105					110			
30	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	
			115					120					125				
35	Phe	Pro	Leu	Ala	Pro	Cys	Ser	Arg	Ser	Thr	Ser	Glu	Ser	Thr	Ala	Ala	
		130					135					140					
40	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	
	145					150					155					160	
45	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	
				165						170					175		
50	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	
				180					185					190			
55	Ser	Ser	Ser	Leu	Gly	Thr	Lys	Thr	Tyr	Thr	Cys	Asn	Val	Asp	His	Lys	
			195					200					205				
60	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Ser	Lys	Tyr	Gly	Pro	
		210					215					220					
65	Pro	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Phe	Leu	Gly	Gly	Pro	Ser	Val	
	225					230					235					240	
70	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	
				245						250					255		
75	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	Gln	Glu	Asp	Pro	Glu	
				260					265					270			

EP 2 424 897 B1

	Val	Gln	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	
				275				280					285				
5	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Phe	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	
		290					295					300					
10	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	
	305					310					315					320	
	Cys	Lys	Val	Ser	Asn	Lys	Gly	Leu	Pro	Ser	Ser	Ile	Glu	Lys	Thr	Ile	
					325					330					335		
15	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	
				340					345					350			
20	Pro	Ser	Gln	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	
			355					360					365				
25	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	
	370						375					380					
	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	
	385					390					395					400	
30	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Arg	Leu	Thr	Val	Asp	Lys	Ser	Arg	
					405					410					415		
35	Trp	Gln	Glu	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	
				420					425					430			
40	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Leu	Gly			
			435					440					445				

<210> 69

<211> 445

<212> PRT

45 <213> Mus musculus

<400> 69

50

55

EP 2 424 897 B1

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

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

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

15 Gly Phe Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr Glu Tyr Ala Ala
50 55 60

15

20

25

30

35

40

45

50

55

EP 2 424 897 B1

	Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ser	Lys	Ser	Ile	65	70	75	80
5	Ala	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Lys	Thr	Glu	Asp	Thr	Ala	Val	Tyr		85	90	95
10	Tyr	Cys	Ala	Arg	Asp	Val	Gly	Ser	Asn	Tyr	Phe	Asp	Tyr	Trp	Gly	Gln		100	105	110
	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val		115	120	125
15	Phe	Pro	Leu	Ala	Pro	Cys	Ser	Arg	Ser	Thr	Ser	Glu	Ser	Thr	Ala	Ala		130	135	140
20	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser		145	150	155
25	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val		165	170	175
	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro		180	185	190
30	Ser	Ser	Asn	Phe	Gly	Thr	Gln	Thr	Tyr	Thr	Cys	Asn	Val	Asp	His	Lys		195	200	205
35	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Thr	Val	Glu	Arg	Lys	Cys	Cys	Val		210	215	220
40	Glu	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Pro	Val	Ala	Gly	Pro	Ser	Val	Phe		225	230	235
	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro		245	250	255
45	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val		260	265	270
50	Gln	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr		275	280	285
	Lys	Pro	Arg	Glu	Glu	Gln	Phe	Asn	Ser	Thr	Phe	Arg	Val	Val	Ser	Val		290	295	300
55	Leu	Thr	Val	Val	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys		305	310	315
																				320

EP 2 424 897 B1

	Lys	Val	Ser	Asn	Lys	Gly	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	
					325					330					335		
5	Lys	Thr	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	
				340					345					350			
10	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	
			355					360					365				
15	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	
		370					375					380					
20	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Met	Leu	Asp	Ser	Asp	
	385					390					395					400	
25	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	
					405					410					415		
30	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	
				420					425					430			
35	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly				
			435					440					445				
40																	
45																	
50																	
55																	

<210> 70
 <211> 219
 <212> PRT
 <213> Mus musculus
 <400> 70

EP 2 424 897 B1

1	Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ala	Val	Ser	Leu	Gly
					5					10					15	
5	Glu	Arg	Ala	Thr	Met	Ser	Cys	Lys	Ser	Ser	Gln	Ser	Leu	Phe	Asn	Ser
				20					25					30		
10	Arg	Thr	Arg	Lys	Asn	Tyr	Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln
			35					40					45			
15	Ser	Pro	Lys	Leu	Leu	Ile	Tyr	Trp	Ala	Ser	Ala	Arg	Asp	Ser	Gly	Val
		50					55					60				
20	Pro	Ala	Arg	Phe	Thr	Gly	Ser	Gly	Ser	Glu	Thr	Tyr	Phe	Thr	Leu	Thr
	65					70					75					80
25	Ile	Ser	Arg	Val	Gln	Ala	Glu	Asp	Leu	Ala	Val	Tyr	Tyr	Cys	Met	Gln
					85					90					95	
30	Ser	Phe	Asn	Leu	Arg	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys
35																
40	Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu
			115					120					125			
45	Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe
		130					135					140				
50	Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln
	145					150					155					160
55	Ser	Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser
					165					170					175	
60	Thr	Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu
				180					185					190		
65	Lys	His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser
			195					200					205			
70	Pro	Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys					
		210						215								
75	<210>	71														
	<211>	219														
	<212>	PRT														
	<213>	Mus musculus														

<400> 71

5	Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Asp	Ser	Leu	Ala	Val	Ser	Leu	Gly
	1				5					10					15	
10	Glu	Arg	Ala	Thr	Met	Ser	Cys	Lys	Ser	Ser	Gln	Ser	Leu	Phe	Asn	Ser
				20					25					30		
15	Arg	Thr	Arg	Lys	Asn	Tyr	Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln
			35					40					45			
20	Pro	Pro	Lys	Leu	Leu	Ile	Tyr	Trp	Ala	Ser	Ala	Arg	Asp	Ser	Gly	Val
		50					55					60				
25	Pro	Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Glu	Thr	Tyr	Phe	Thr	Leu	Thr
	65					70					75					80
30	Ile	Ser	Arg	Val	Gln	Ala	Glu	Asp	Leu	Ala	Val	Tyr	Tyr	Cys	Met	Gln
					85					90					95	
35	Ser	Phe	Asn	Leu	Arg	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys
				100					105					110		
40	Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu
			115					120					125			
45	Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe
		130					135					140				
50	Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln
	145					150					155					160
55	Ser	Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser
					165					170					175	
60	Thr	Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu
				180					185					190		
65	Lys	His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser
			195					200					205			
70	Pro	Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys					
		210					215									

55 <210> 72
<211> 360
<212> DNA
<213> Mus musculus

EP 2 424 897 B1

<400> 72

	gaggtgcagc	tggtggagtc	tggcggagga	ctggtgcagc	ccggcagaag	cctgagactg	60
5	agctgcaccg	ccagcggctt	caccttcacc	gacaactaca	tgtcctgggt	gcgccaggcc	120
	cctggaaaagg	gcctggaatg	ggtgggcttc	atccggaaca	aggccaacgg	ctacaccaca	180
	gagtacgccg	ccagcgtgaa	gggccgggttc	accatcagcc	gggacaacag	caagagcatt	240
10	gcctacctgc	agatgaacag	cctgaaaaac	gaggacaccg	ccgtgtacta	ctgcgccagg	300
	gacgtgggca	gcaactactt	cgactactgg	ggccagggca	cactggtgac	cgtgtctagc	360

15
 <210> 73
 <211> 336
 <212> DNA
 <213> Mus musculus

20
 <400> 73

	gacatcgtga	tgacccagag	ccccagcagc	ctggccgtgt	ctctgggcga	gcggggccacc	60
25	atgagctgca	agagcagcca	gagcctgttc	aacagccgga	cccgaagaa	ctacctggcc	120
	tggtatcagc	agaagcccgg	ccagtcccc	aagctgctga	tctactgggc	cagcgccaga	180
	gatagcggcg	tgcccgcctcg	ctttaccggc	agcggcagcg	agacctactt	caccctgacc	240
30	atcagccggg	tgcaggccga	ggacctcgcc	gtgtactact	gcatgcagag	cttcaacctg	300
	cggaccttcg	gccagggcac	caaggtggag	atcaag			336

35
 <210> 74
 <211> 336
 <212> DNA
 <213> Mus musculus

40
 <400> 74

	gacatcgtga	tgacccagtc	ccccgaactc	ctggccgtgt	ctctgggcga	gcggggccacc	60
	atgtcctgca	agtcctccca	gtccctgttc	aactcccgga	cccgaagaa	ctacctggcc	120
45	tggtatcagc	agaagcccgg	ccagcccccc	aagctgctga	tctactgggc	ctctgctaga	180
	gactctggcg	tgcccagacag	attctccggc	tccggcagcg	agacatactt	caccctgacc	240
	atctcccggg	tgcaggccga	ggatctggcc	gtgtactact	gcatgcagtc	cttcaacctg	300
50	cggaccttcg	gccagggcac	caaggtggaa	atcaag			336

55
 <210> 75
 <211> 1341
 <212> DNA
 <213> Mus musculus

<400> 75

EP 2 424 897 B1

	gaggtgcagc tgggtggagtc tggcggagga ctggtgcagc ccggcagaag cctgagactg	60
	agctgcaccg ccagcggctt caccttcacc gacaactaca tgtcctgggt gcgccaggcc	120
5	cctggaaagg gcctggaatg ggtgggcttc atccggaaca aggccaacgg ctacaccaca	180
	gagtacgccg ccagcgtgaa gggccggctt accatcagcc gggacaacag caagagcatt	240
	gcctacctgc agatgaacag cctgaaaacc gaggacaccg ccgtgtacta ctgcgccagg	300
10	gacgtgggca gcaactactt cgactactgg ggccagggca cactggtgac cgtgtctagc	360
	gccagcacca agggcccaag cgtgttcccc ctggccccct gctccagaag caccagcgag	420
	agcacagccg ccctgggctg cctggtgaag gactacttcc ccgagcccg gaccgtgtcc	480
15	tggaaacagcg gagccctgac cagcggcgtg cacaccttcc ccgccgtgct gcagagcagc	540
	ggcctgtaca gcctgagcag cgtggtgacc gtgccagca gcagcctggg caccaagacc	600
20	tacacctgta acgtggacca caagcccagc aacaccaagg tggacaagag ggtggagagc	660
	aagtacggcc caccctgccc cagctgccc acccccgagt tcctgggcgg acccagcgtg	720
	ttcctgttcc cccccaagcc caaggacacc ctgatgatca gcagaacccc cgaggtgacc	780
25	tgtgtggtgg tggacgtgtc ccaggaggac cccgaggtcc agttcaactg gtacgtggac	840
	ggcgtggagg tgcacaacgc caagaccaag ccagagagg agcagtttaa cagcacctac	900
30	cgggtggtgt ccgtgctgac cgtgctgcac caggactggc tgaacggcaa agagtacaag	960
	tgtaaaggtct ccaacaaggg cctgccaaagc agcatcgaaa agaccatcag caaggccaag	1020
	ggccagccta gagagcccca ggtctacacc ctgccaccca gccaaagagga gatgaccaag	1080
35	aaccaggtgt ccctgacctg tctggtgaag ggcttctacc caagcgacat cgccgtggag	1140
	tgggagagca acggccagcc cgagaacaac tacaagacca cccccagc gctggacagc	1200
40	gacggcagct tcttcctgta cagcaggctg accgtggaca agtccagatg gcaggagggc	1260
	aacgtcttta gctgctccgt gatgcacgag gccctgcaca accactacac ccagaagagc	1320
	ctgagcctgt ccctgggctg a	1341
45	<210> 76 <211> 1341 <212> DNA <213> Mus musculus	
50	<400> 76	
55		

	gaggtgcagc tgggtggagtc tggcggagga ctggtgcagc ccggcagaag cctgagactg	60
	agctgcaccg ccagcggcctt caccttcacc gacaactaca tgtcctgggt gcgccaggcc	120
5	cctggaaagg gcctggaatg ggtgggcttc atccggaaca aggccaacgg ctacaccaca	180
	gagtacgccg ccagcgtgaa gggccggcttc accatcagcc gggacaacag caagagcatt	240
10	gcctacctgc agatgaacag cctgaaaacc gaggacaccg ccgtgtacta ctgcgccagg	300
	gacgtgggca gcaactactt cgactactgg ggccagggca cactggtgac cgtgtctagc	360
	gccagcacca agggcccaag cgtgttcccc ctggccccct gctccagaag caccagcgag	420
15	agcacagccg ccctgggctg cctggtgaag gactacttcc ccgagcccgt gaccgtgtcc	480
	tggaaacagcg gagccctgac cagcggcgtg cacaccttcc ccgccgtgct gcagagcagc	540
	ggcctgtaca gcctgagcag cgtggtgacc gtgccagca gcagcctggg caccaagacc	600
20	tacacctgta acgtggacca caagcccagc aacaccaagg tggacaagag ggtggagagc	660
	aagtacggcc caccctgccc cccctgccc acccccgagt tcctgggagg acccagcgtg	720
25	ttcctgttcc cccccaagcc caaggacacc ctgatgatca gcagaacccc cgaggtgacc	780
	tgtgtggtgg tggacgtgtc ccaggaggac ccgaggtcc agttcaactg gtacgtggac	840
	ggcgtggagg tgcacaacgc caagaccaag ccagagagg agcagtttaa cagcacctac	900
30	cgggtggtgt ccgtgctgac cgtgctgcac caggactggc tgaacggcaa agagtacaag	960
	tgtaaggtct ccaacaaggg cctgccaaag agcatcgaaa agaccatcag caaggccaag	1020
	ggccagccta gagagcccca ggtctacacc ctgccaccca gccaaagagga gatgaccaag	1080
35	aaccaggtgt ccctgacctg tctggtgaag ggcttctacc caagcgacat cgccgtggag	1140
	tgggagagca acggccagcc cgagaacaac tacaagacca cccccccagt gctggacagc	1200
	gacggcagct tcttcctgta cagcaggctg accgtggaca agtccagatg gcaggagggc	1260
40	aacgtcttta gctgctccgt gatgcacgag gccctgcaca accactacac ccagaagagc	1320
	ctgagcctgt ccctgggctg a	1341
45	<210> 77 <211> 1338 <212> DNA <213> Mus musculus	
50	<400> 77	
55		

	gaggtgcagc tggtggagtc tggcggagga ctggtgcagc ccggcagaag cctgagactg	60
	agctgcaccg ccagcggcctt caccttcacc gacaactaca tgtcctgggt gcgccaggcc	120
5	cctggaaagg gcctggaatg ggtgggcttc atccggaaca aggccaacgg ctacaccaca	180
	gagtagcccg ccagcgtgaa gggccggctt accatcagcc gggacaacag caagagcatt	240
10	gcctacctgc agatgaacag cctgaaaacc gaggacaccg ccgtgtacta ctgcgccagg	300
	gacgtgggca gcaactactt cgactactgg ggccagggca cactgggtgac cgtgtctagc	360
	gccagcacca agggcccaag cgtgttcccc ctggccccct gctccagaag caccagcgag	420
15	agcacagccg ccctgggctg cctggtgaag gactacttcc ccgagcccgt gaccgtgtcc	480
	tggaaacagcg gagccctgac cagcggcgtg cacaccttcc ccgccgtgct gcagagcagc	540
	ggcctgtaca gcctgagcag cgtgggtgacc gtgccaaagca gcaacttcgg caccagacc	600
20	tacacctgta acgtggacca caagcccagc aacaccaagg tggacaagac cgtggagagg	660
	aagtgtgtg tggagtgcc cccctgcca gccccccag tggccggacc cagcgtgttc	720
25	ctgttcccc ccaagcccaa ggacacctg atgatcagca gaacccccga ggtgacctgt	780
	gtggtggtgg acgtgtccca cgaggacccc gaggtgcagt tcaactggta cgtggacggc	840
	gtggaggtgc acaacgcca gaccaagccc agagaggagc agtttaacag caccttcgg	900
30	gtggtgtccg tgctgaccgt ggtgcaccag gactggctga acggcaagga gtacaagtgt	960
	aaggtctcca acaagggcct gccagcccc atcgaaaaga ccatcagcaa gaccaaggga	1020
	cagccaagag agccacaggt ctacacctg cccccagca gggaggagat gaccaagaac	1080
35	caggtgtccc tgacctgtct ggtgaagggc ttctacccaa gcgacatcgc cgtggagtgg	1140
	gagagcaacg gccagcccg gaacaactac aagaccaccc ccccaatgct ggacagcgac	1200
40	ggcagcttct tcctgtacag caagctgaca gtggacaaga gcagatggca gcagggaac	1260
	gtgttcagct gctccgtgat gcacgaggcc ctgcacaacc actacacca gaagagcctg	1320
	agcctgtccc caggctga	1338
45	<210> 78 <211> 660 <212> DNA <213> Mus musculus	
50	<400> 78	
55		

EP 2 424 897 B1

	gacatcgtga tgacccagag cccagcagc ctggccgtgt ctctgggcga gcgggccacc	60
	atgagctgca agagcagcca gagcctgttc aacagccgga cccggaagaa ctacctggcc	120
5	tggtatcagc agaagcccgg ccagtcctcc aagctgctga tctactgggc cagcgccaga	180
	gatagcggcg tgcccgcctcg ctttaccggc agcggcagcg agacctactt caccctgacc	240
	atcagccggg tgcaggccga ggacctcgcc gtgtactact gcatgcagag cttcaacctg	300
10	cggaccttcg gccagggcac caaggtggag atcaagcgta cggcggccgc tcccagcgtg	360
	ttcatcttcc cccaagcga cgagcagctg aagagcggca ccgccagcgt ggtgtgtctg	420
15	ctgaacaact tctaccccag ggaggccaag gtgcagtgga aggtggacaa cgccctgcag	480
	agcggcaaca gccaggagag cgtcaccgag caggacagca aggactccac ctacagcctg	540
	agcagcacc tgacctgag caaggccgac tacgagaagc acaaggtgta cgcctgtgag	600
20	gtgaccacc aggccctgtc cagccccgtg accaagagct tcaacagggg cgagtgtga	660
	<210> 79	
	<211> 660	
25	<212> DNA	
	<213> Mus musculus	
	<400> 79	
30	gacatcgtga tgacccagtc ccccgactcc ctggccgtgt ctctgggcga gcgggccacc	60
	atgtcctgca agtcctccca gtccctgttc aactcccgga cccggaagaa ctacctggcc	120
	tggtatcagc agaagcccgg ccagcccccc aagctgctga tctactgggc ctctgctaga	180
35	gactctggcg tgcccgcagc attctccggc tccggcagcg agacatactt caccctgacc	240
	atctcccggg tgcaggccga ggatctggcc gtgtactact gcatgcagtc cttcaacctg	300
	cggaccttcg gccagggcac caaggtggaa atcaagcgta cggcggccgc tcccagcgtg	360
	ttcatcttcc cccaagcga cgagcagctg aagagcggca ccgccagcgt ggtgtgtctg	420
	ctgaacaact tctaccccag ggaggccaag gtgcagtgga aggtggacaa cgccctgcag	480
45	agcggcaaca gccaggagag cgtcaccgag caggacagca aggactccac ctacagcctg	540
	agcagcacc tgacctgag caaggccgac tacgagaagc acaaggtgta cgcctgtgag	600
	gtgaccacc aggccctgtc cagccccgtg accaagagct tcaacagggg cgagtgtga	660
50	<210> 80	
	<211> 450	
	<212> PRT	
	<213> Mus musculus	
55	<400> 80	

EP 2 424 897 B1

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

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

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

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

15

20

25

30

35

40

45

50

55

EP 2 424 897 B1

	Ser	Val	Arg	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ser	Gln	Ser	Ile	65	70	75	80
5	Leu	Tyr	Leu	Gln	Met	Asn	Ala	Leu	Arg	Ala	Glu	Asp	Ser	Ala	Thr	Tyr	85	90	95	
10	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	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	115	120	125	
15	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	130	135	140	
20	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	145	150	155	160
25	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	165	170	175	
	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	180	185	190	
30	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	195	200	205	
35	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp	210	215	220	
40	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	225	230	235	240
	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	245	250	255	
45	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	260	265	270	
50	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	275	280	285	
	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	290	295	300	
55	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	305	310	315	320

EP 2 424 897 B1

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
 325 330 335
 5 Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
 340 345 350
 10 Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
 355 360 365
 15 Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
 370 375 380
 20 Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
 385 390 395 400
 25 Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
 405 410 415
 30 Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
 420 425 430
 35 Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
 435 440 445
 40 Gly Lys
 450

<210> 81
 <211> 1353
 <212> DNA
 <213> Mus musculus

<400> 81

EP 2 424 897 B1

	gaagtgaacc tgggtggagtc tggcggcgga ctggtgcagc ctgggggag cctgagactg	60
	agctgcgcca cctccggctt caccttcacc gacaactaca tgagctgggt gcgccagccc	120
5	cctggcaagg ccctggaatg gctgggcttc atccggaaca aggccaacgg ctacaccacc	180
	gactacagcg ccagcgtgcg gggcagattc accatcagcc gggacaacag ccagagcatc	240
	ctgtacctgc agatgaacgc cctgcggggc gaggacagcg ccacctacta ctgtgcccgg	300
10	gacgtgggca gcaactactt cgactactgg ggccagggca ccacactgac cgtgtccagc	360
	gccagcacca agggcccctc cgtgttcccg ctagcccca gcagcaagag caccagcggc	420
	ggcacagccg ccctgggctg cctggtgaag gactacttcc ccgagcccg gaccgtgtcc	480
15	tggaacagcg gagccctgac ctccggcgtg cacaccttcc ccgccgtgct gcagagcagc	540
	ggcctgtaca gcctgagcag cgtggtgacc gtgcccagca gcagcctggg caccagacc	600
20	tacatctgta acgtgaacca caagcccagc aacaccaagg tggacaagag agtggagccc	660
	aagagctgtg acaagaccca cacctgcccc ccctgccag ccccgagct gctgggcgga	720
	cccagcgtgt tcctgttccc ccccaagccc aaggacaccc tgatgatcag cagaaccccc	780
25	gaggtgacct gtgtggtggt ggacgtgtcc cagcaggacc cagaggtgaa gttcaactgg	840
	tacgtggacg gcgtggaggt gcacaacgcc aagaccaagc ccagagagga gcagtacaac	900
30	agcacctaca ggggtggtgtc cgtgctgacc gtgctgcacc aggactggct gaacggcaag	960
	gagtacaagt gtaaggtgtc caacaaggcc ctgccagccc caatcgaaa gaccatcagc	1020
	aaggccaagg gccagccaag agagccccag gtgtacaccc tgccaccag cagggaggag	1080
35	atgaccaaga accaggtgtc cctgacctgt ctggtgaagg gcttctaccc aagcgacatc	1140
	gccgtggagt gggagagcaa cggccagccc gagaacaact acaagaccac ccccccagtg	1200
	ctggacagcg acggcagctt cttcctgtac agcaagctga ccgtggacaa gagcagatgg	1260
40	cagcagggca acgtgttcag ctgctccgtg atgcacgagg ccctgcacaa ccactacacc	1320
	cagaagagcc tgagcctgtc ccagggcaag tga	1353
45	<210> 82 <211> 112 <212> PRT <213> Mus musculus	
50	<400> 82	
55		

EP 2 424 897 B1

1	Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Asp	Ser	Leu	Ala	Val	Ser	Leu	Gly
	1				5					10					15	
5	Glu	Arg	Ala	Thr	Met	Ser	Cys	Lys	Ser	Ser	Gln	Ser	Leu	Phe	Asn	Ser
				20					25					30		
10	Arg	Thr	Arg	Lys	Asn	Tyr	Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln
			35					40					45			
15	Pro	Pro	Lys	Leu	Leu	Ile	Tyr	Trp	Ala	Ser	Ala	Arg	Asp	Ser	Gly	Val
		50					55					60				
20	Pro	Asp	Arg	Phe	Thr	Gly	Ser	Gly	Ser	Glu	Thr	Tyr	Phe	Thr	Leu	Thr
	65					70					75					80
25	Ile	Ser	Arg	Val	Gln	Ala	Glu	Asp	Val	Ala	Val	Tyr	Tyr	Cys	Met	Gln
					85					90					95	
30	Ser	Phe	Asn	Leu	Arg	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys
				100					105					110		
	<210> 83															
	<211> 112															
	<212> PRT															
	<213> Mus musculus															
	<400> 83															
35	Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Asp	Ser	Leu	Ala	Val	Ser	Leu	Gly
	1				5					10					15	
40	Glu	Arg	Ala	Thr	Met	Ser	Cys	Lys	Ser	Ser	Gln	Ser	Leu	Phe	Asn	Ser
				20					25					30		
45	Arg	Thr	Arg	Lys	Asn	Tyr	Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln
			35					40					45			
50	Pro	Pro	Lys	Leu	Leu	Ile	Tyr	Trp	Ala	Ser	Ala	Arg	Asp	Ser	Gly	Val
		50					55					60				
55	Pro	Asp	Arg	Phe	Thr	Gly	Ser	Gly	Ser	Glu	Thr	Tyr	Phe	Thr	Leu	Thr
	65					70					75					80
	Ile	Ser	Ser	Leu	Gln	Ala	Glu	Asp	Leu	Ala	Val	Tyr	Tyr	Cys	Met	Gln
				85						90					95	
	Ser	Phe	Asn	Leu	Arg	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys
				100					105					110		

<210> 84
 <211> 219
 <212> PRT
 <213> Mus musculus

5

<400> 84

10

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

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

15

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

20

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

25

Pro Asp 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 Val Ala Val Tyr Tyr Cys Met Gln
 85 90 95

30

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

35

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu

40

45

50

55

EP 2 424 897 B1

	115	120	125
5	Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe 130 135 140		
10	Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln 145 150 155 160		
15	Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser 165 170 175		
20	Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu 180 185 190		
25	Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser 195 200 205		
30	Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys 210 215		
35	<210> 85 <211> 219 <212> PRT <213> Mus musculus		
40			
45			
50			
55			

EP 2 424 897 B1

1	Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Asp	Ser	Leu	Ala	Val	Ser	Leu	Gly
					5				10						15	
5	Glu	Arg	Ala	Thr	Met	Ser	Cys	Lys	Ser	Ser	Gln	Ser	Leu	Phe	Asn	Ser
				20					25					30		
10	Arg	Thr	Arg	Lys	Asn	Tyr	Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln
			35					40					45			
15	Pro	Pro	Lys	Leu	Leu	Ile	Tyr	Trp	Ala	Ser	Ala	Arg	Asp	Ser	Gly	Val
			50				55					60				
20	Pro	Asp	Arg	Phe	Thr	Gly	Ser	Gly	Ser	Glu	Thr	Tyr	Phe	Thr	Leu	Thr
	65					70					75					80
25	Ile	Ser	Ser	Leu	Gln	Ala	Glu	Asp	Leu	Ala	Val	Tyr	Tyr	Cys	Met	Gln
					85					90					95	
30	Ser	Phe	Asn	Leu	Arg	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys
				100					105					110		
35	Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu
			115					120					125			
40	Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe
			130				135					140				
45	Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln
	145					150					155					160
50	Ser	Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser
					165					170					175	
55	Thr	Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu
				180					185					190		
60	Lys	His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser
			195					200					205			
65	Pro	Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys					
			210				215									

<210> 86

<211> 336

<212> DNA

<213> Mus musculus

<400> 86

EP 2 424 897 B1

	gacatcgtga tgacccagtc ccccgactcc ctggccgtgt ctctgggcga gcgggccacc	60
	atgtcctgca agtcctccca gtccctgttc aactcccga cccggaagaa ctacctggcc	120
5	tggtatcagc agaagcccgg ccagcccccc aagctgctga tctactgggc ctctgctaga	180
	gactctggcg tgcccagacag attcaccggc tccggcagcg agacatactt caccctgacc	240
	atctcccggg tgcaggccga ggatgtggcc gtgtactact gcatgcagtc cttcaacctg	300
10	cggaccttcg gccagggcac caaggtggaa atcaag	336
	<210> 87	
	<211> 336	
15	<212> DNA	
	<213> Mus musculus	
	<400> 87	
20	gacatcgtga tgacccagtc ccccgactcc ctggccgtgt ctctgggcga gcgggccacc	60
	atgtcctgca agtcctccca gtccctgttc aactcccga cccggaagaa ctacctggcc	120
	tggtatcagc agaagcccgg ccagcccccc aagctgctga tctactgggc ctctgctaga	180
25	gactctggcg tgcccagacag attcaccggc tccggcagcg agacatactt caccctgacc	240
	atctccagcc tgcaggccga ggatctggcc gtgtactact gcatgcagtc cttcaacctg	300
30	cggaccttcg gccagggcac caaggtggaa atcaag	336
	<210> 88	
	<211> 657	
	<212> DNA	
35	<213> Mus musculus	
	<400> 88	
40	gacatcgtga tgacccagtc ccccgactcc ctggccgtgt ctctgggcga gcgggccacc	60
	atgtcctgca agtcctccca gtccctgttc aactcccga cccggaagaa ctacctggcc	120
	tggtatcagc agaagcccgg ccagcccccc aagctgctga tctactgggc ctctgctaga	180
	gactctggcg tgcccagacag attcaccggc tccggcagcg agacatactt caccctgacc	240
45	atctcccggg tgcaggccga ggatgtggcc gtgtactact gcatgcagtc cttcaacctg	300
	cggaccttcg gccagggcac caaggtggaa atcaagcgta cggaggccgc tcccagcgtg	360
50	ttcatcttcc cccaagcga cgagcagctg aagagcggca ccgccagcgt ggtgtgtctg	420
	ctgaacaact tctaccccag ggaggccaag gtgcagtggg aggtggacaa cgccctgcag	480
	agcggcaaca gccaggagag cgtcaccgag caggacagca aggactccac ctacagcctg	540
55	agcagcacc tgaccctgag caaggccgac tacgagaagc acaaggtgta cgcctgtgag	600
	gtgaccacc aggccctgtc cagccccgtg accaagagct tcaacagggg cgagtgc	657

EP 2 424 897 B1

<210> 89
 <211> 657
 <212> DNA
 <213> Mus musculus

5
 <400> 89

gacatcgtga tgacccagtc ccccgactcc ctggccgtgt ctctgggcga gcggggccacc 60
 10 atgtcctgca agtcctccca gtccctgttc aactcccgga cccggaagaa ctacctggcc 120
 tggatatcagc agaagcccgg ccagcccccc aagctgctga tctactgggc ctctgctaga 180
 gactctggcg tgcccagacag attcacccggc tccggcagcg agacataactt caccctgacc 240
 15 atctccagcc tgcaggccga ggatctggcc gtgtactact gcatgcagtc cttcaacctg 300
 cggaccttcg gccagggcac caaggtggaa atcaagcgta cggcggccgc tcccagcgtg 360
 20 ttcacatctcc cccaagcga cgagcagctg aagagcggca ccgccagcgt ggtgtgtctg 420
 ctgaacaact tctaccccag ggaggccaag gtgcagtgga aggtggacaa cgccctgcag 480
 agcggcaaca gccaggagag cgtcacccgag caggacagca aggactccac ctacagcctg 540
 25 agcagcaccg tgaccctgag caaggccgac tacgagaagc acaaggtgta cgcctgtgag 600
 gtgacccacc agggcctgtc cagccccgtg accaagagct tcaacagggg cgagtgc 657

30
 <210> 90
 <211> 120
 <212> PRT
 <213> Mus musculus
 <400> 90

35
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg
 1 5 10 15

40

45

50

55

EP 2 424 897 B1

Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Thr Asp Asn
 20 25 30
 5 Tyr Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 10 Gly Phe Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr Glu Tyr Ala Ala
 50 55 60
 15 Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Ile
 65 70 75 80
 20 Ala Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
 85 90 95
 25 Tyr Cys Ala Arg Asp Val Gly Ser Asn Tyr Phe Asp Tyr Trp Gly Gln
 100 105 110
 30 Gly Thr Leu Val Thr Val Ser Ser
 115 120
 <210> 91
 <211> 360
 <212> DNA
 <213> Mus musculus
 <400> 91
 35 gaggtgcagc tgggtggagtc tggcggagga ctggtgcagc ccggcagaag cctgagactg 60
 agctgcaccg ccagcggctt caccttcacc gacaactaca tgtcctgggt gcgccaggcc 120
 cctggaaagg gcctggaatg ggtgggcttc atccggaaca aggccaacgg ctacaccaca 180
 40 gagtacgccg ccagcgtgaa gggccgggttc accatcagcc gggacgacag caagagcatt 240
 gcctacctgc agatgaacag cctgaaaacc gaggacaccg ccgtgtacta ctgcgccagg 300
 gacgtgggca gcaactactt cgactactgg ggccagggca cactggtgac cgtgtctagc 360
 45 <210> 92
 <211> 450
 <212> PRT
 <213> Mus musculus
 50 <400> 92
 55

EP 2 424 897 B1

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

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

10 Tyr Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val

15

20

25

30

35

40

45

50

55

EP 2 424 897 B1

	35	40	45
5	Gly Phe Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr Glu Tyr Ala Ala 50 55 60		
	Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Ile 65 70 75 80		
10	Ala Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr 85 90 95		
15	Tyr Cys Ala Arg Asp Val Gly Ser Asn Tyr Phe Asp Tyr Trp Gly Gln 100 105 110		
	Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val 115 120 125		
20	Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala 130 135 140		
25	Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser 145 150 155 160		
	Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val 165 170 175		
30	Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro 180 185 190		
35	Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys 195 200 205		
	Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp 210 215 220		
40	Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly 225 230 235 240		
45	Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile 245 250 255		
	Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu 260 265 270		
50	Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His 275 280 285		
55	Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg 290 295 300		

EP 2 424 897 B1

	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys
	305					310					315					320
5	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu
					325					330					335	
10	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr
				340					345					350		
15	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu
			355					360					365			
20	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp
	370						375					380				
25	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val
	385					390					395					400
30	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp
					405					410					415	
35	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His
				420					425					430		
40	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro
			435					440					445			
45	Gly	Lys														
			450													

<210> 93

<211> 1350

<212> DNA

<213> Mus musculus

<400> 93

gaggtgcagc tgggtggagtc tggcggagga ctggtgcagc ccggcagaag cctgagactg 60
 agctgcaccg ccagcggcctt caccttcacc gacaactaca tgtcctgggt gcgccaggcc 120
 5 cctggaaagg gcctggaatg ggtgggcttc atccggaaca aggccaacgg ctacaccaca 180
 gagtacgccg ccagcgtgaa gggccgggttc accatcagcc gggacgacag caagagcatt 240
 gcctacctgc agatgaacag cctgaaaacc gaggacaccg ccgtgtacta ctgcgccagg 300
 10 gacgtgggca gcaactactt cgactactgg gggcagggca cactgggtgac cgtgtctagc 360
 gccagcaciaa agggcccaag cgtgttcccg ctagcccccga gcagcaagag caccagcggc 420
 15 ggcacagccg ccctgggctg cctggtgaag gactacttcc ccgagcccgt gaccgtgtcc 480
 tggaacagcg gagccctgac ctccggcgtg cacaccttcc ccgccgtgct gcagagcagc 540
 ggctgtaca gcctgagcag cgtggtgacc gtgccagca gcagcctggg caccagacc 600
 20 tacatctgta acgtgaacca caagcccagc aacaccaagg tggacaagag agtggagccc 660
 aagagctgtg acaagaccca cacctgcccc ccctgccag ccccgagct gctgggcgga 720
 25 cccagcgtgt tcctgttccc ccccaagccc aaggacacc tgatgatcag cagaaccccc 780
 gaggtgacct gtgtgggtggg ggacgtgtcc cagcaggacc cagaggtgaa gttcaactgg 840
 tacgtggacg gcgtggagggt gcacaacgcc aagaccaagc ccagagagga gcagtacaac 900
 30 agcacctaca ggggtggtgtc cgtgctgacc gtgctgcacc aggactggct gaacggcaag 960
 gagtacaagt gtaagggtgtc caacaaggcc ctgccagccc caatcgaaaa gaccatcagc 1020
 35 aaggccaagg gccagccaag agagccccag gtgtacaccc tgccaccag cagggaggag 1080
 atgaccaaga accaggtgtc cctgacctgt ctggtgaagg gcttctaccc aagcgacatc 1140
 gccgtggagt gggagagcaa cggccagccc gagaacaact acaagaccac cccccagtg 1200
 40 ctggacagcg acggcagctt ctctctgtac agcaagctga ccgtggacaa gagcagatgg 1260
 cagcagggca acgtgttcag ctgctccgtg atgcacgagg ccctgcaciaa ccactacacc 1320
 cagaagagcc tgagcctgtc cccaggcaag 1350
 45

Claims

1. An isolated anti-CXCR4 antibody, or one of its functional fragments, said functional fragment being selected among the fragments Fv, Fab, (Fab')₂, Fab', scFv, scFv-Fc and diabodies, **characterized in that** it comprises:

- a light chain comprising CDR-L1, CDR-L2 and CDR-L3 comprising respectively the amino acid sequence SEQ ID Nos. 1, 2 and 3; and a heavy chain comprising CDR-H1, CDR-H2 and CDR-H3 comprising respectively the amino acid sequence SEQ ID Nos. 4, 5 and 6; or
 - a light chain comprising CDR-L1, CDR-L2 and CDR-L3 comprising respectively the amino acid sequence SEQ ID Nos. 1, 2 and 30; and a heavy chain comprising CDR-H1, CDR-H2 and CDR-H3 comprising respectively the amino acid sequence SEQ ID Nos. 31, 32 and 33.

2. The antibody of claim 1, or a functional fragment of same, **characterized in that** said antibody is:

- an antibody comprising a light chain comprising the amino acid sequence SEQ ID No. 7 and a heavy chain comprising the amino acid sequence SEQ ID No. 8 or
- an antibody comprising a light chain comprising the amino acid sequence SEQ ID No. 34, and a heavy chain comprising the amino acid sequence SEQ ID No. 35.

3. The antibody of claim 1, or a functional fragment of same, **characterized in that** it is a chimeric antibody and **in that** it comprises a heavy chain of sequence selected from the group consisting of SEQ ID Nos. 56, 57 or 58, and a light chain of sequence SEQ ID No. 59.

4. The antibody of claim 1, **characterized in that** it is a humanized antibody and **in that** it comprises a heavy chain variable region of sequence consisting of SEQ ID No. 64, and a light chain variable region of sequence selected from the group consisting of SEQ ID Nos. 65, 66, 82 or 83.

5. The antibody of claim 1, **characterized in that** it is a humanized antibody and **in that** it comprises a humanized antibody, or a functional fragment of same, comprising a heavy chain of sequence selected from the group consisting of SEQ ID Nos. 67, 68 or 69, and a light chain of sequence selected from the group consisting of SEQ ID Nos. 70, 71, 84 or 85.

6. The antibody of claim 1, **characterized in that** said functional fragment consists of a fragment scFv, said scFv comprising the amino acid sequence SEQ ID No. 54.

7. An isolated nucleic acid, **characterized in that** it is a nucleic acid, DNA or RNA, coding for an antibody, or one of its functional fragments, as claimed in one of claims 1 to 6.

8. A vector comprising a nucleic acid as claimed in claim 7.

9. A host cell comprising a vector as claimed in claim 8.

10. A process for production of an antibody, or one of its functional fragments, as claimed in one of claims 1 to 6 **characterized in that** it comprises the following stages:

- a) culture in a medium and appropriate culture conditions of a cell as claimed in claim 9; and
- b) the recovery of said antibodies, or one of their functional fragments, thus produced starting from the culture medium or said cultured cells.

11. The antibody of claims 1 to 6 for use as a medicament.

12. The antibody, or one of its functional fragments, of claims 1 to 6 **characterized in that** it inhibits HIV-1 KON primary isolate replication in PBMC with an IC₉₀ of at least 5 µg/ml, preferably at least 10 µg/ml.

13. A pharmaceutical composition comprising by way of active principle a compound consisting of an antibody, or one of its functional fragments, as claimed in one of claims 1 to 6 and 12.

14. The composition as claimed in claim 13 for the prevention or for the treatment of HIV infection.

15. The composition as claimed in claim 14, **characterized in that** said HIV infection is a X4-tropic HIV infection, and/or a X4/R5-tropic HIV infection.

16. The composition according to claims 13 to 15, **characterized in that** it comprises at least a second anti-HIV compound selected in the group consisting of antiretroviral drugs such as HIV protease inhibitors (PI), nucleoside /nucleotide HIV reverse-transcriptase inhibitors (NRTI/NtRTI), non-nucleoside HIV reverse-transcriptase inhibitors (NNRTI), HIV entry inhibitors, HIV integrase inhibitors.

17. The composition according to claim 15 or 16, **characterized in that** said at least second anti-HIV compound is the Maraviroc.

18. Process for the screening and/or the identification of molecules as CXCR4 antagonist antiviral agents comprising the steps of:

- a) selecting cells expressing CXCR4,
- b) incubating said cells with an antibody, or one of its functional fragments or derivatives, of claims 1 to 6 or 12, and
- c) evaluating the tested molecules for their potential inhibition of the binding between the antibody, or one of its functional fragments or derivatives, to CXCR4, and
- d) selecting molecules capable of said inhibition.

Patentansprüche

1. Isolierter anti-CXCR4-Antikörper oder eines von dessen funktionalen Fragmenten, wobei das funktionale Fragment aus den Fragmenten Fv, Fab, (Fab')₂, Fab', scFv, scFv-Fc und Diabodies ausgewählt ist, **dadurch gekennzeichnet, dass** er bzw. es umfasst:

- eine leichte Kette, umfassend CDR-L1, CDR-L2 und CDR-L3, umfassend jeweils die Aminosäuresequenz SEQ ID Nr. 1, 2 bzw. 3; und eine schwere Kette, umfassend CDR-H1, CDR-H2 und CDR-H3, umfassend jeweils die Aminosäuresequenz SEQ ID Nr. 4, 5 und 6; oder
- eine leichte Kette, umfassend CDR-L1, CDR-L2 und CDR-L3, umfassend jeweils die Aminosäuresequenz SEQ ID Nr. 1, 2 bzw. 30; und eine schwere Kette, umfassend CDR-H1, CDR-H2 und CDR-H3, umfassend jeweils die Aminosäuresequenz SEQ ID Nr. 31, 32 bzw. 33.

2. Antikörper nach Anspruch 1 oder ein funktionales Fragment von diesem, **dadurch gekennzeichnet, dass** der Antikörper:

- ein Antikörper, umfassend eine leichte Kette, welche die Aminosäuresequenz SEQ ID Nr. 7 umfasst, und eine schwere Kette, welche die Aminosäuresequenz SEQ ID Nr. 8 umfasst, oder
- ein Antikörper, umfassend eine leichte Kette, welche die Aminosäuresequenz SEQ ID Nr. 34 umfasst, und eine schwere Kette, welche die Aminosäuresequenz SEQ ID Nr. 35 umfasst,

ist.

3. Antikörper nach Anspruch 1 oder ein funktionales Fragment von diesem, **dadurch gekennzeichnet, dass** er bzw. es ein chimärer Antikörper ist und dass er bzw. es eine schwere Kette mit einer Sequenz, ausgewählt aus der Gruppe bestehend aus den SEQ ID Nr. 56, 57 oder 58, und eine leichte Kette mit der Sequenz SEQ ID Nr. 59 umfasst.

4. Antikörper nach Anspruch 1, **dadurch gekennzeichnet, dass** er ein humanisierter Antikörper ist und dass er eine variable Region einer schweren Kette mit einer Sequenz bestehend aus SEQ ID Nr. 64 und eine variable Region einer leichten Kette mit einer Sequenz, ausgewählt aus der Gruppe bestehend aus den SEQ ID Nr. 65, 66, 82 oder 83, umfasst.

5. Antikörper nach Anspruch 1, **dadurch gekennzeichnet, dass** er ein humanisierter Antikörper ist und dass er einen humanisierten Antikörper umfasst, oder ein funktionales Fragment von diesem, umfassend eine schwere Kette mit einer Sequenz, ausgewählt aus der Gruppe bestehend aus den SEQ ID Nr. 67, 68 oder 69, und eine leichte Kette mit einer Sequenz, ausgewählt aus der Gruppe bestehend aus den SEQ ID Nr. 70, 71, 84 oder 85.

6. Antikörper nach Anspruch 1, **dadurch gekennzeichnet, dass** das funktionale Fragment aus einem scFv-Fragment besteht, wobei das scFv die Aminosäuresequenz SEQ ID Nr. 54 umfasst.

7. Isolierte Nukleinsäure, **dadurch gekennzeichnet, dass** sie eine Nukleinsäure, DNA oder RNA ist, welche einen Antikörper oder eines von dessen funktionalen Fragmenten, wie in einem der Ansprüche 1 bis 6 beansprucht, kodiert.

8. Vektor, welcher eine Nukleinsäure, wie in Anspruch 7 beansprucht, umfasst.

9. Wirtszelle, welche einen Vektor, wie in Anspruch 8 beansprucht, umfasst.

10. Verfahren zur Herstellung eines Antikörpers oder von einem von dessen funktionalen Fragmenten, wie in einem

der Ansprüche 1 bis 6 beansprucht, **dadurch gekennzeichnet, dass** es die folgenden Schritte umfasst:

- Kultur einer Zelle, wie in Anspruch 9 beansprucht, in einem Medium und unter geeigneten Kulturbedingungen; und
- die Gewinnung der Antikörper oder von einem von deren funktionalen Fragmenten, die dementsprechend produziert worden sind, ausgehend von dem Kulturmedium oder den kultivierten Zellen.

11. Antikörper nach den Ansprüchen 1 bis 6 zur Verwendung als ein Arzneimittel.

12. Antikörper oder eines von dessen funktionalen Fragmenten nach den Ansprüchen 1 bis 6, **dadurch gekennzeichnet, dass** er bzw. es eine Replikation von HIV-1-KON-Primärisolat in PBMC mit einer IC₅₀ von mindestens 5 µg/ml, vorzugsweise mindestens 10 µg/ml inhibiert.

13. Pharmazeutische Zusammensetzung, umfassend als Wirkstoff eine Verbindung bestehend aus einem Antikörper oder einem von dessen funktionalen Fragmenten, wie in einem der Ansprüche 1 bis 6 und 12 beansprucht.

14. Zusammensetzung, wie in Anspruch 13 beansprucht, für die Verhütung oder für die Behandlung einer HIV-Infektion.

15. Zusammensetzung, wie in Anspruch 14 beansprucht, **dadurch gekennzeichnet, dass** die HIV-Infektion eine X4-trope HIV-Infektion und/oder eine X4/R5-trope HIV-Infektion ist.

16. Zusammensetzung nach den Ansprüchen 13 bis 15, **dadurch gekennzeichnet, dass** sie mindestens eine zweite anti-HIV-Verbindung, ausgewählt aus der Gruppe bestehend aus antiretroviralen Arzneimitteln, wie HIV-Protease-Inhibitoren (PI), Nukleosid/Nukleotid-HIV-reverse Transkriptase-Inhibitoren (NRTI/NtRTI), nicht-Nukleosid-HIV-reverse Transkriptase-Inhibitoren (NNRTI), HIV-Eintritts-Inhibitoren, HIV-Integrase-Inhibitoren, umfasst.

17. Zusammensetzung nach Anspruch 15 oder 16, **dadurch gekennzeichnet, dass** die mindestens zweite anti-HIV-Verbindung Maraviroc ist.

18. Verfahren zum Screenen und/oder zur Identifizierung von Molekülen als CXCR4-antagonistisch wirkende antivirale Mittel, umfassend die Schritte:

- a) Selektieren von Zellen, die CXCR4 exprimieren;
- b) Inkubieren der Zellen mit einem Antikörper oder einem von dessen funktionalen Fragmenten oder Derivaten nach den Ansprüchen 1 bis 6 oder 12 und
- c) Auswerten der getesteten Moleküle hinsichtlich ihrer potentiellen Inhibition der Bindung des Antikörpers oder von einem von dessen funktionalen Fragmenten oder Derivaten an CXCR4 und
- d) Selektieren von Molekülen, die zu der Inhibition in der Lage sind.

Revendications

1. Anticorps anti-CXCR4 isolé, ou l'un de ses fragments fonctionnels, ledit fragment fonctionnel étant choisi parmi les fragments Fv, Fab, (Fab')₂, Fab', scFv, scFv-Fc et les diabodies, **caractérisé en ce qu'il** comprend :

- une chaîne légère comprenant les CDR-L1, CDR-L2 et CDR-L3 comprenant respectivement la séquence d'acides aminés SEQ ID NO : 1, 2 et 3 ; et une chaîne lourde comprenant les CDR-H1, CDR-H2 et CDR-H3 comprenant respectivement la séquence d'acides aminés SEQ ID NO : 4, 5 et 6 ; ou
- une chaîne légère comprenant les CDR-L1, CDR-L2 et CDR-L3 comprenant respectivement la séquence d'acides aminés SEQ ID NO : 1, 2 et 30 ; et une chaîne lourde comprenant les CDR-H1, CDR-H2 et CDR-H3 comprenant respectivement la séquence d'acides aminés SEQ ID NO : 31, 32 et 33.

2. Anticorps selon la revendication 1, ou un fragment fonctionnel de celui-ci, **caractérisé en ce que** ledit anticorps est :

- un anticorps comprenant une chaîne légère comprenant la séquence d'acides aminés SEQ ID NO : 7 et une chaîne lourde comprenant la séquence d'acides aminés SEQ ID NO : 8 ou
- un anticorps comprenant une chaîne légère comprenant la séquence d'acides aminés SEQ ID NO : 34 et une chaîne lourde comprenant la séquence d'acides aminés SEQ ID NO : 35.

3. Anticorps selon la revendication 1, **caractérisé en ce qu'il** s'agit d'un anticorps chimérique et **en ce qu'il** comprend une chaîne lourde de séquence choisie dans le groupe constitué par SEQ ID NO : 56, 57 ou 58, et une chaîne légère de séquence SEQ ID NO : 59.
- 5 4. Anticorps selon la revendication 1, **caractérisé en ce qu'il** s'agit d'un anticorps humanisé et **en ce qu'il** comprend une région variable de chaîne lourde de séquence constituée de SEQ ID NO : 64, et une région variable de chaîne légère de séquence choisie dans le groupe constitué par SEQ ID NO : 65, 66, 82 ou 83.
- 10 5. Anticorps selon la revendication 1, **caractérisé en ce qu'il** s'agit d'un anticorps humanisé et **en ce qu'il** comprend un anticorps humanisé, ou un fragment fonctionnel de celui-ci, comprenant une chaîne lourde de séquence choisie dans le groupe constitué par SEQ ID NO : 67, 68 ou 69, et une chaîne légère de séquence choisie dans le groupe constitué par SEQ ID NO : 70, 71, 84 ou 85.
- 15 6. Anticorps selon la revendication 1, **caractérisé en ce que** ledit fragment fonctionnel est constitué d'un fragment scFv, ledit scFv comprenant la séquence d'acides aminés SEQ ID NO : 54.
7. Acide nucléique isolé, **caractérisé en ce qu'il** s'agit d'un acide nucléique, ADN ou ARN, codant un anticorps, ou l'un de ses fragments fonctionnels, selon l'une des revendications 1 à 6.
- 20 8. Vecteur comprenant un acide nucléique selon la revendication 7.
9. Cellule hôte comprenant un vecteur selon la revendication 8.
- 25 10. Procédé de production d'un anticorps, ou de l'un de ses fragments fonctionnels, selon l'une des revendications 1 à 6, **caractérisé en ce qu'il** comprend les étapes suivantes :
 - a) la culture dans un milieu et dans des conditions de culture appropriées d'une cellule hôte selon la revendication 9 ; et
 - b) la récupération desdits anticorps, ou d'un de leurs fragments fonctionnels, ainsi produits à partir du milieu de culture ou desdites cellules cultivées.
- 30 11. Anticorps selon les revendications 1 à 6, pour son utilisation en tant que médicament.
- 35 12. Anticorps, ou un de ses fragments fonctionnels, selon les revendications 1 à 6 **caractérisé en ce qu'il** inhibe la réplication de l'isolat primaire KON du VIH-1 dans des CMSP avec une CI_{90} d'au moins 5 µg/ml, de préférence au moins 10 µg/ml.
- 40 13. Composition pharmaceutique comprenant à titre de principe actif un composé constitué d'un anticorps, ou d'un de ses fragments fonctionnels, selon l'une des revendications 1 à 6 et 12.
- 45 14. Composition selon la revendication 13 pour la prévention ou pour le traitement d'une infection par le VIH.
15. Composition selon la revendication 14, **caractérisée en ce que** ladite infection par le VIH est une infection par VIH X4-tropique, et/ou une infection par VIH X4/R5-tropique.
- 50 16. Composition selon les revendications 13 à 15, **caractérisée en ce qu'elle** comprend au moins un second composé anti-VIH choisi dans le groupe constitué de médicaments antirétroviraux tels que des inhibiteurs de la protéase du VIH (IP), des inhibiteurs nucléosidiques/nucléotidiques de la transcriptase inverse du VIH (INTI/INTTI), des inhibiteurs non nucléosidiques de la transcriptase inverse du VIH (INNTI), des inhibiteurs d'entrée du VIH, des inhibiteurs de l'intégrase du VIH.
- 55 17. Composition selon la revendication 15 ou 16, **caractérisée en ce que** ledit au moins un second composé anti-VIH est le Maraviroc.
18. Procédé de criblage et/ou d'identification de molécules en tant qu'agents antiviraux antagonistes du CXCR4 comprenant les étapes suivantes :
 - a) la sélection de cellules exprimant le CXCR4,

EP 2 424 897 B1

b) l'incubation desdites cellules avec un anticorps, ou l'un de ses fragments ou dérivés fonctionnels, selon les revendications 1 à 6 ou 12, et

c) l'évaluation des molécules testées pour leur inhibition potentielle de la liaison entre l'anticorps, ou l'un de ses fragments ou dérivés fonctionnels, et le CXCR4, et

d) la sélection des molécules capables de ladite inhibition.

5

10

15

20

25

30

35

40

45

50

55

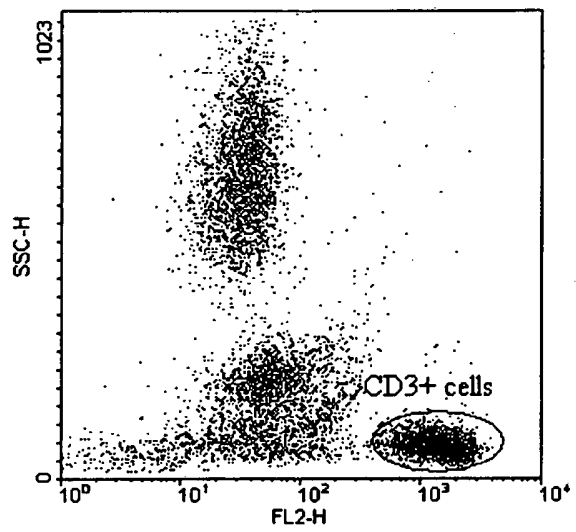


FIGURE 1A

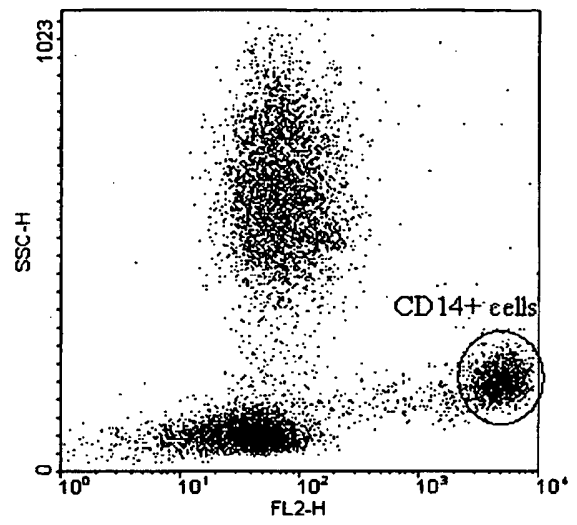


FIGURE 1B

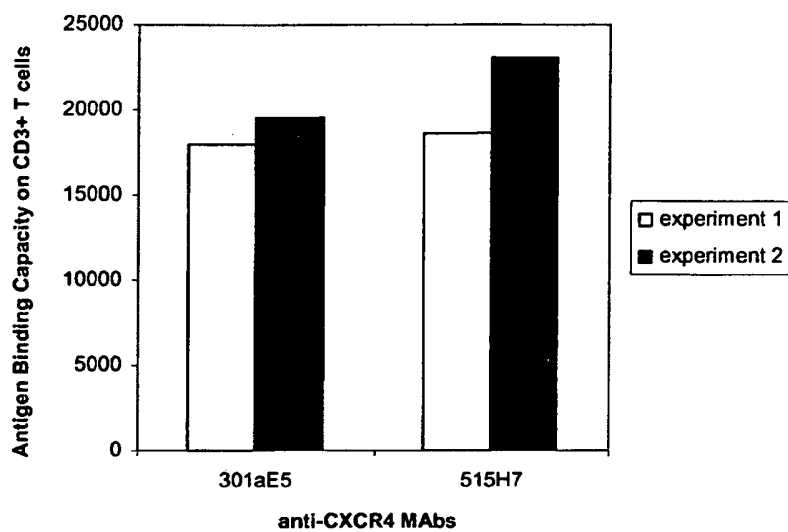


FIGURE 2A

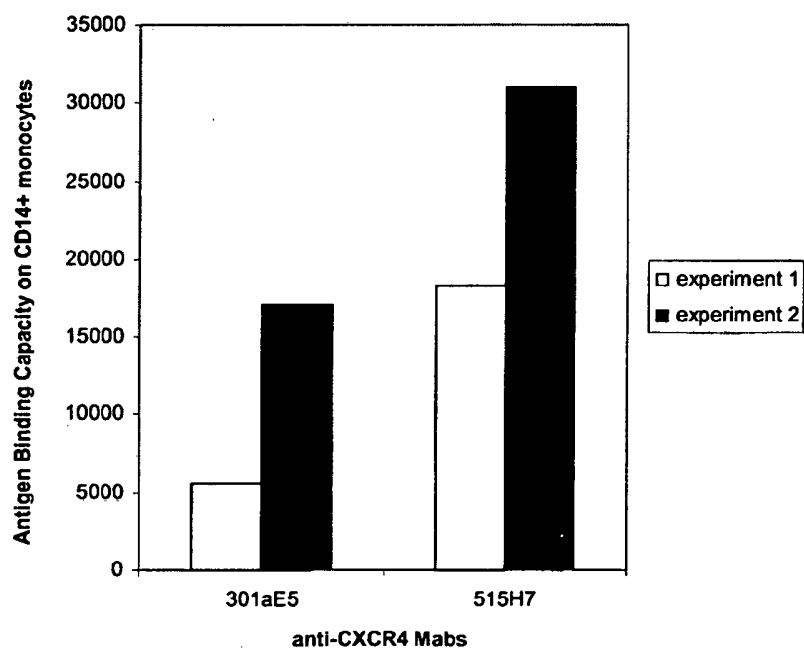


FIGURE 2B

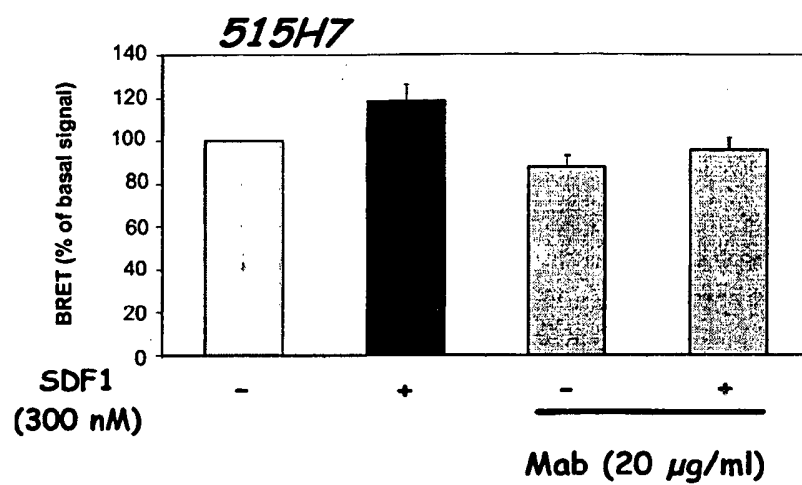


FIGURE 3A

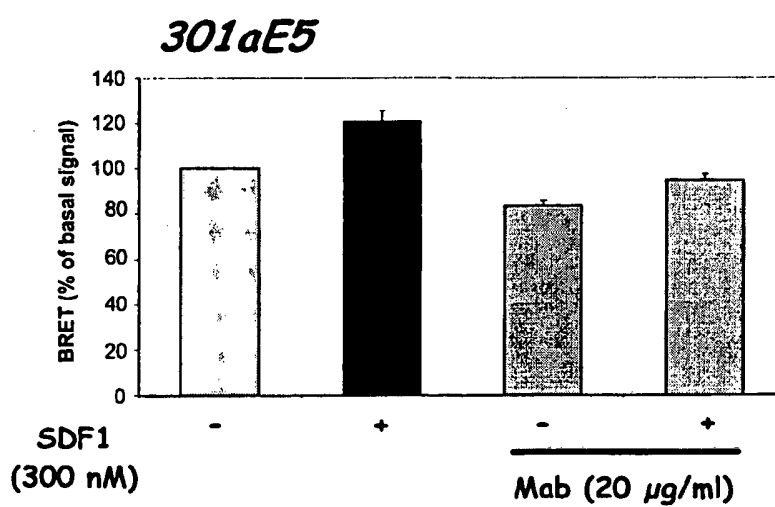


FIGURE 3B

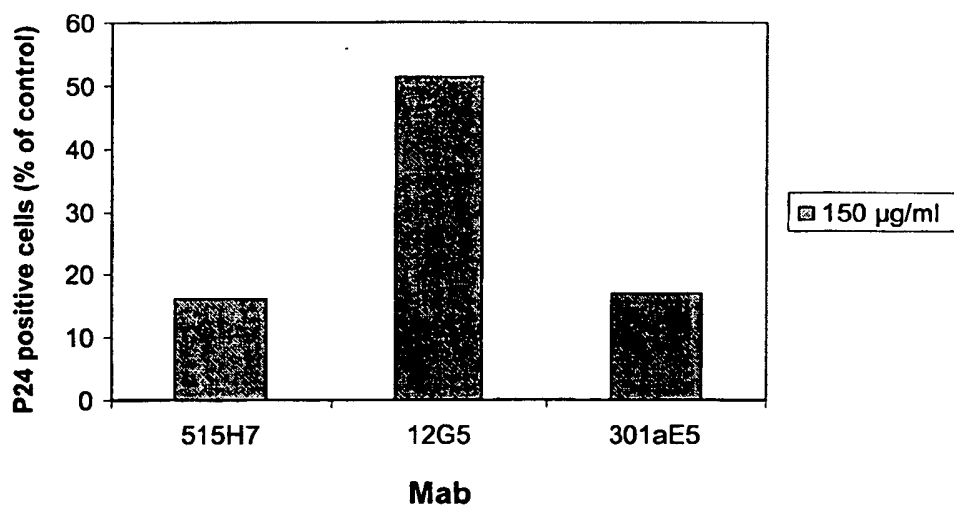


FIGURE 4A

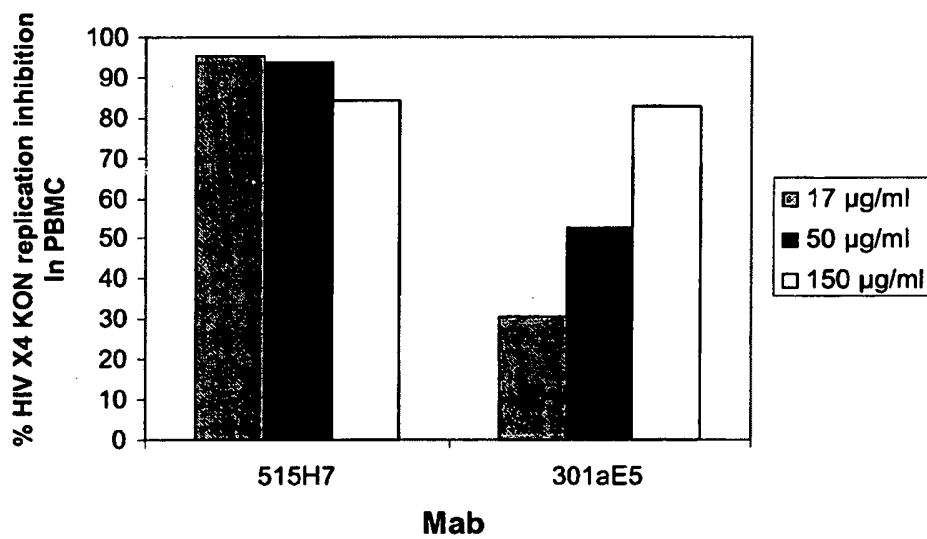


FIGURE 4B

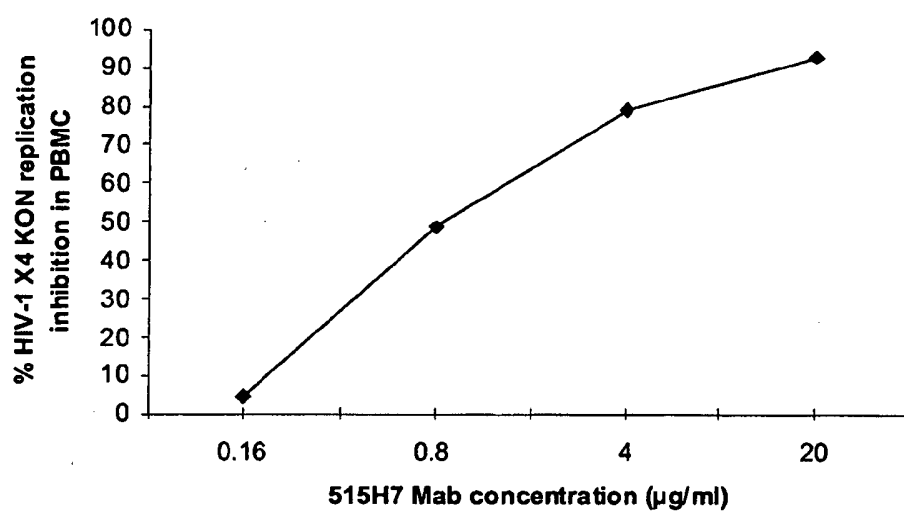


FIGURE 5

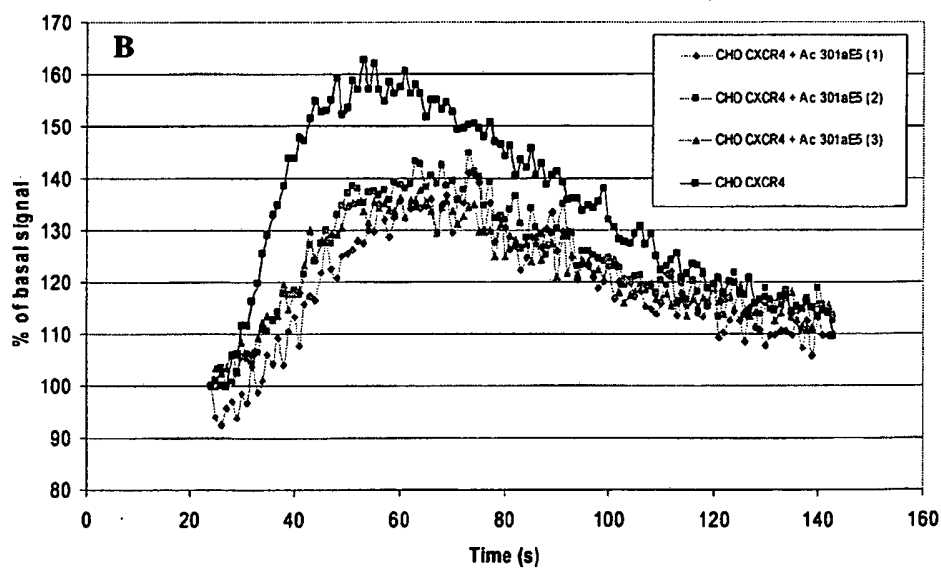
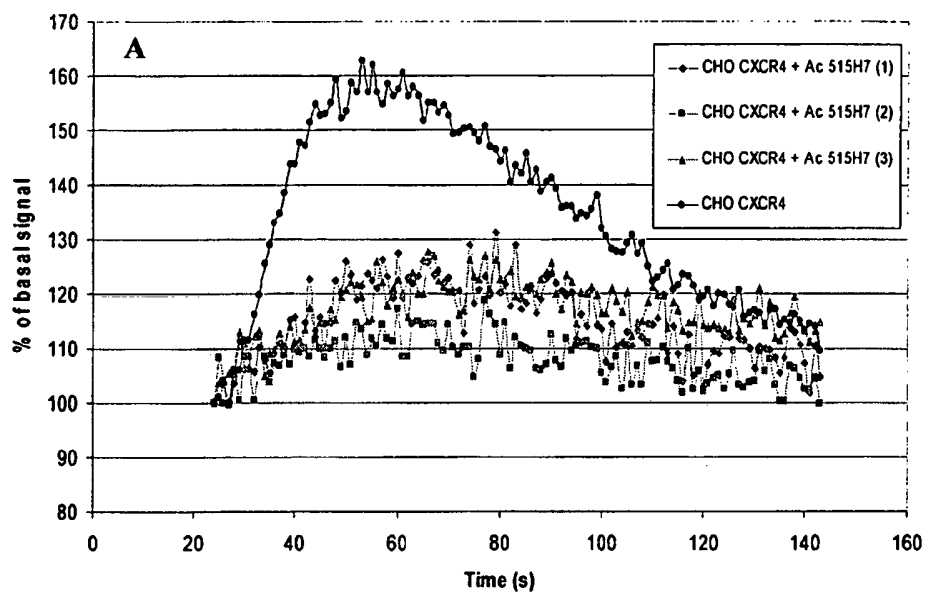


FIGURE 6A et 6B

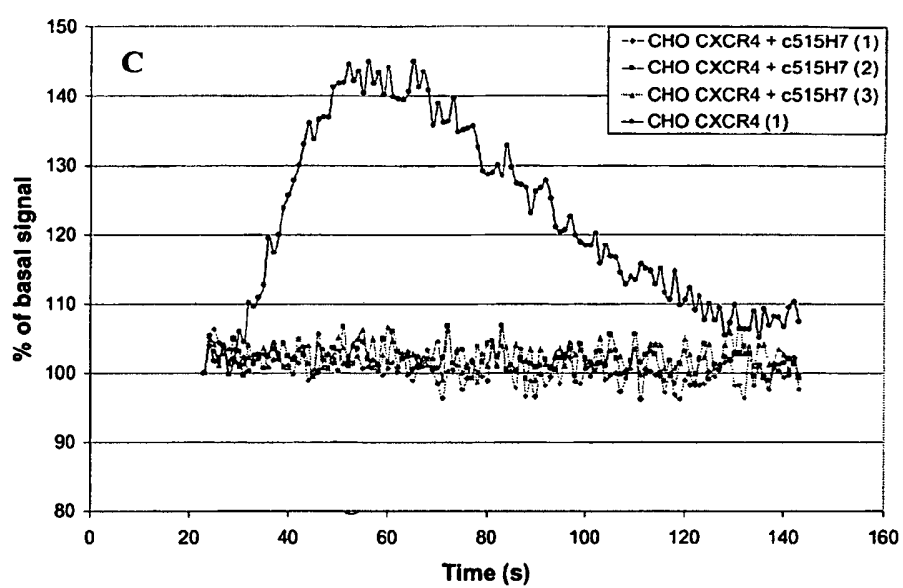


FIGURE 6C

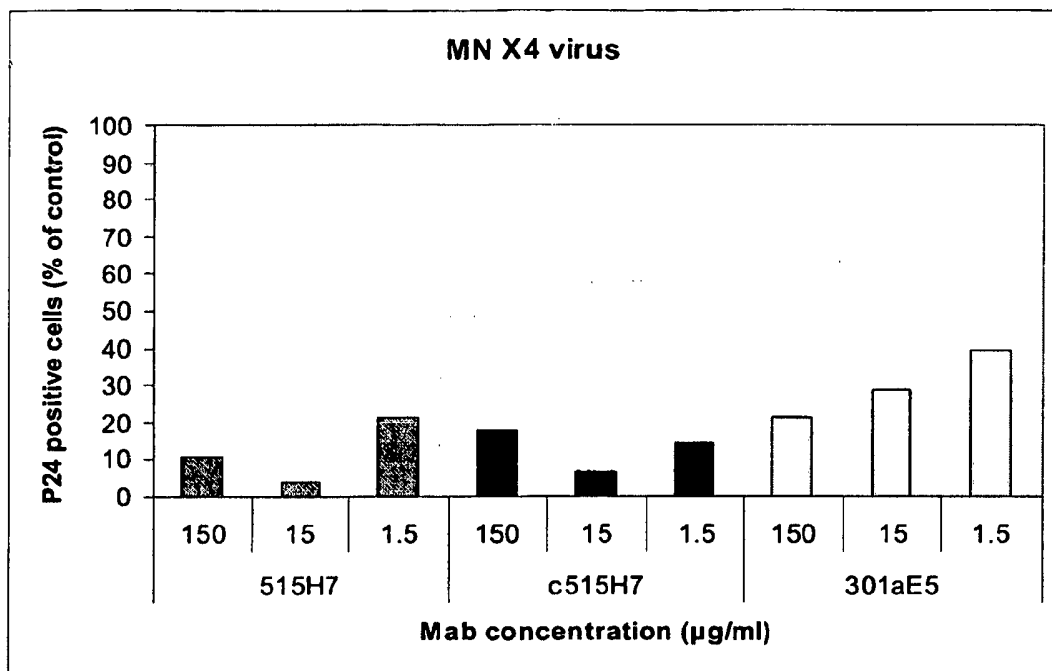


Figure 7

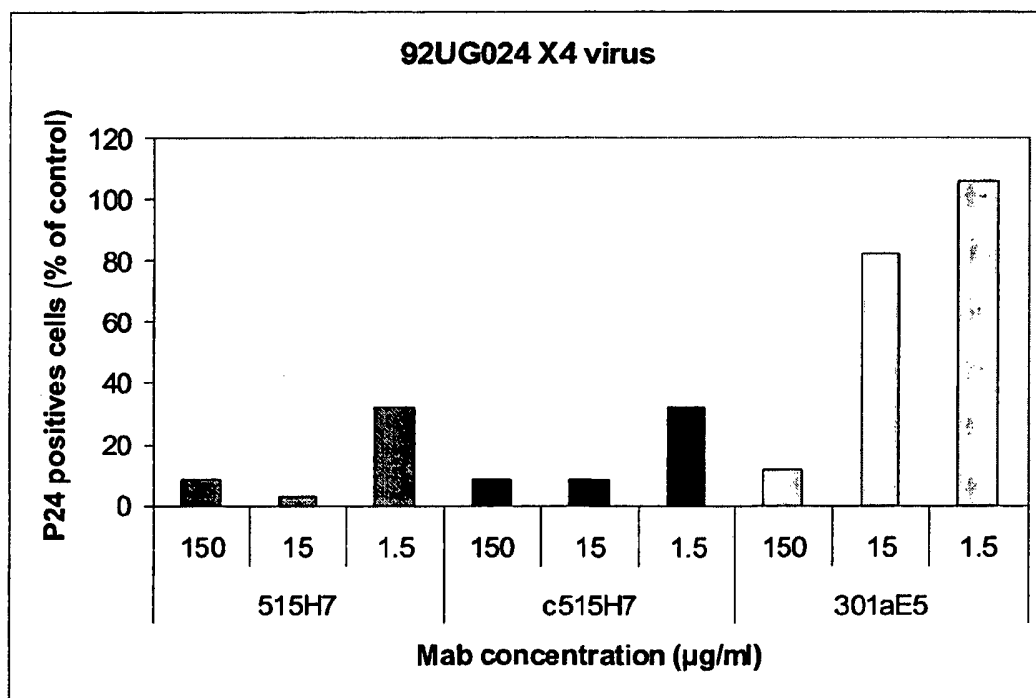


Figure 8

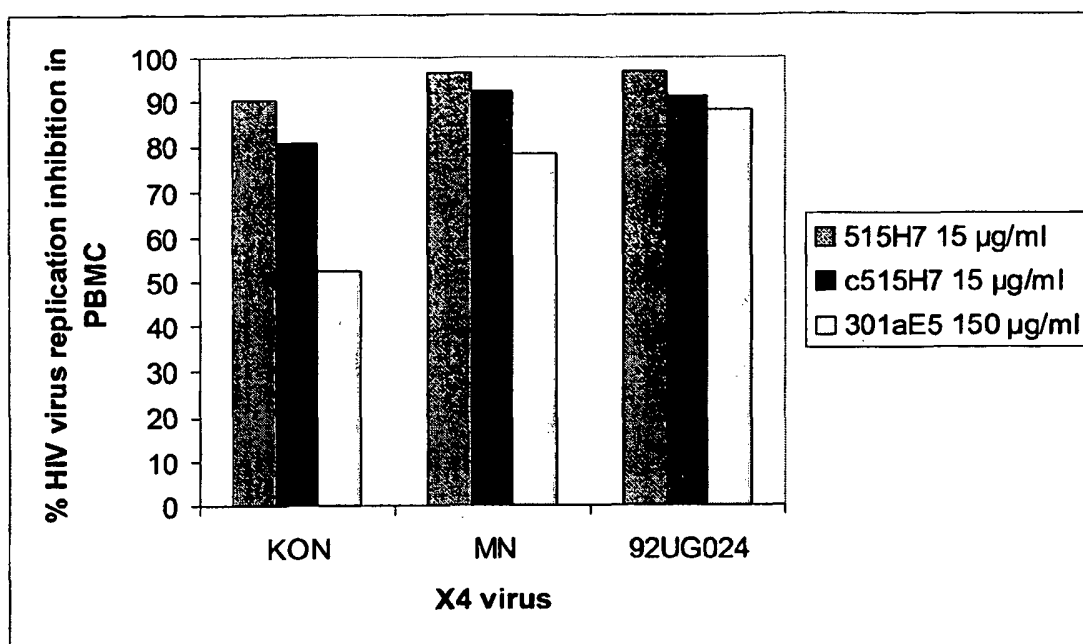


Figure 9

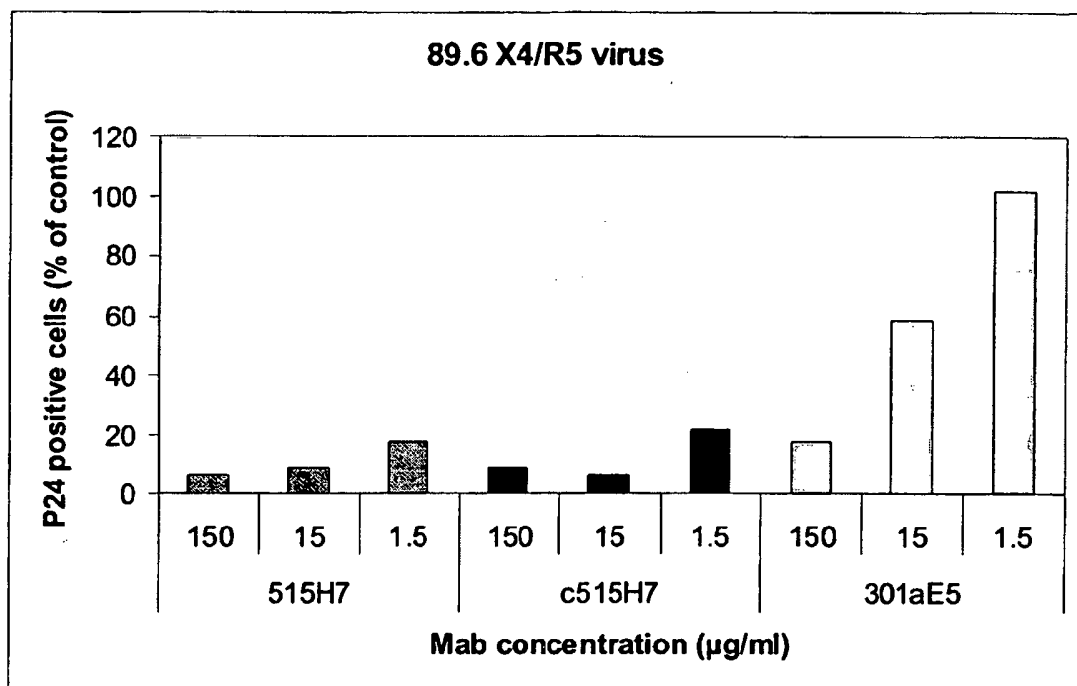


Figure 10

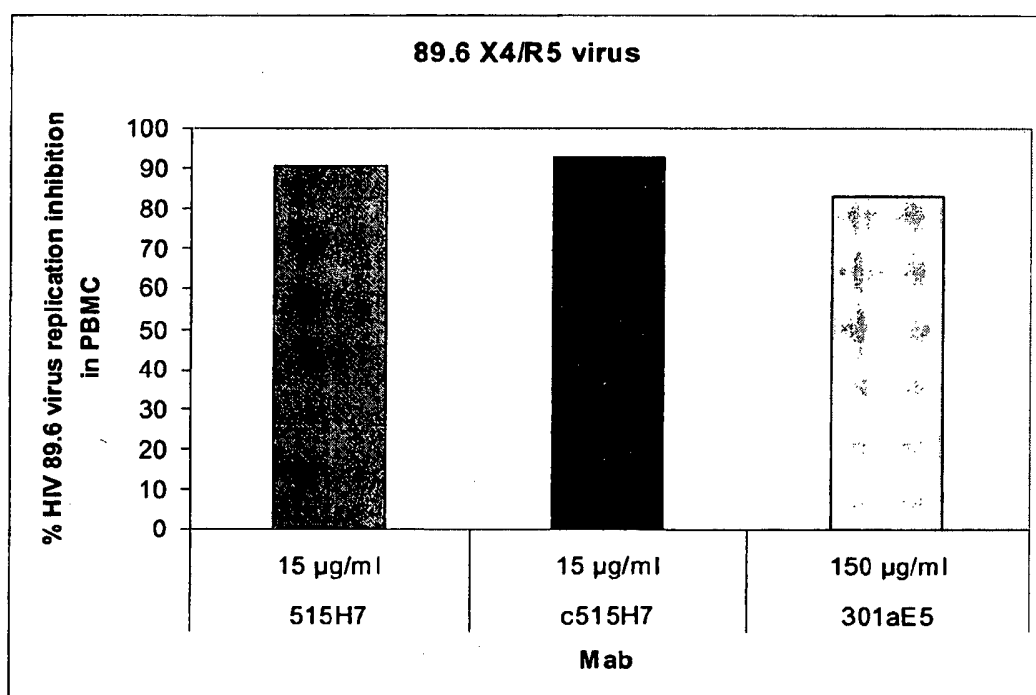


Figure 11

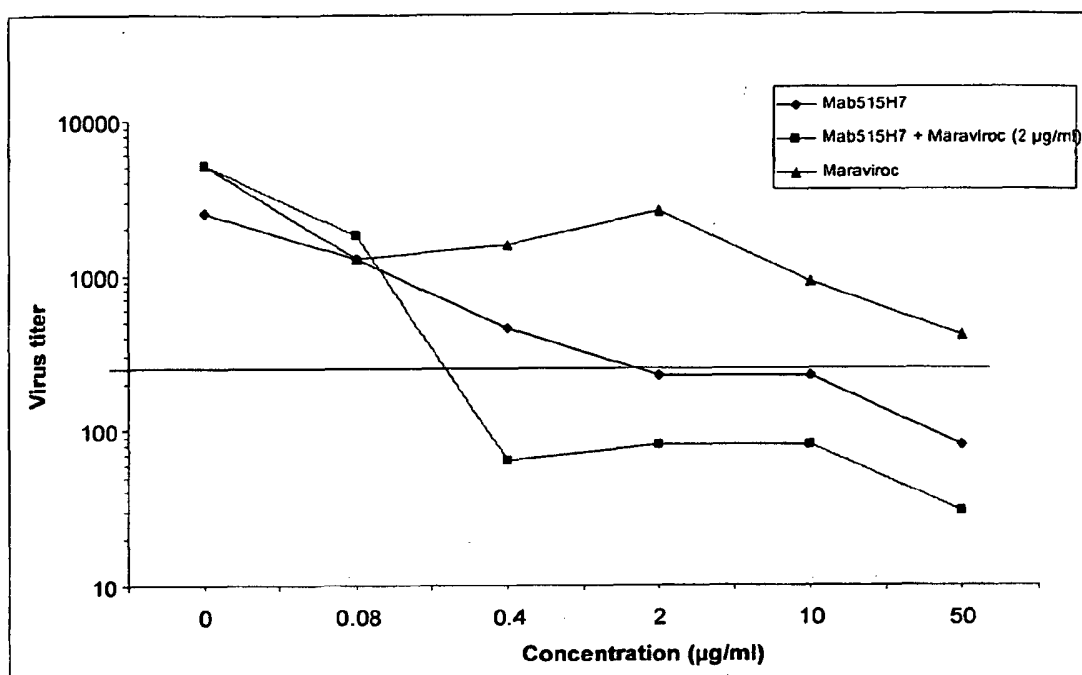


Figure 12

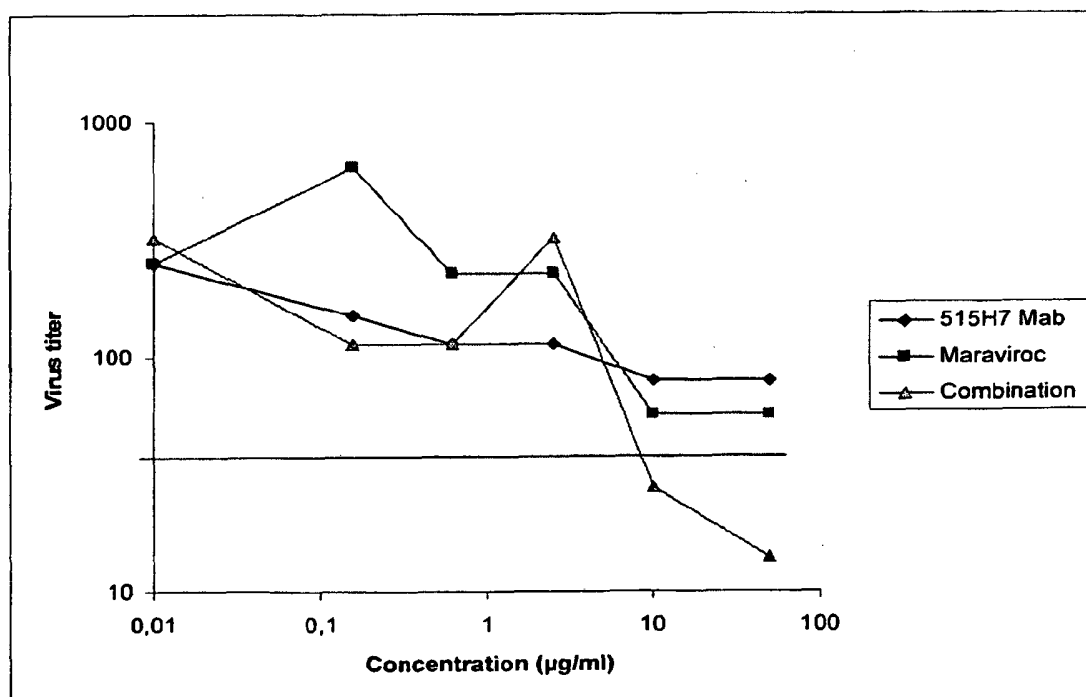


Figure 13

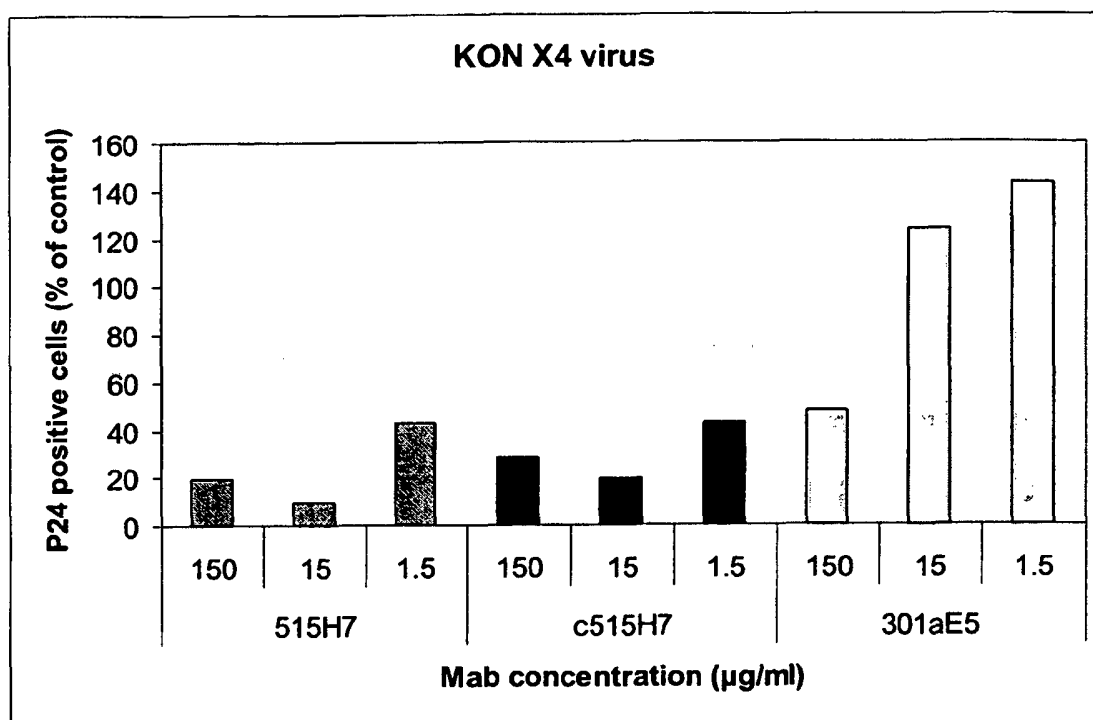


Figure 14

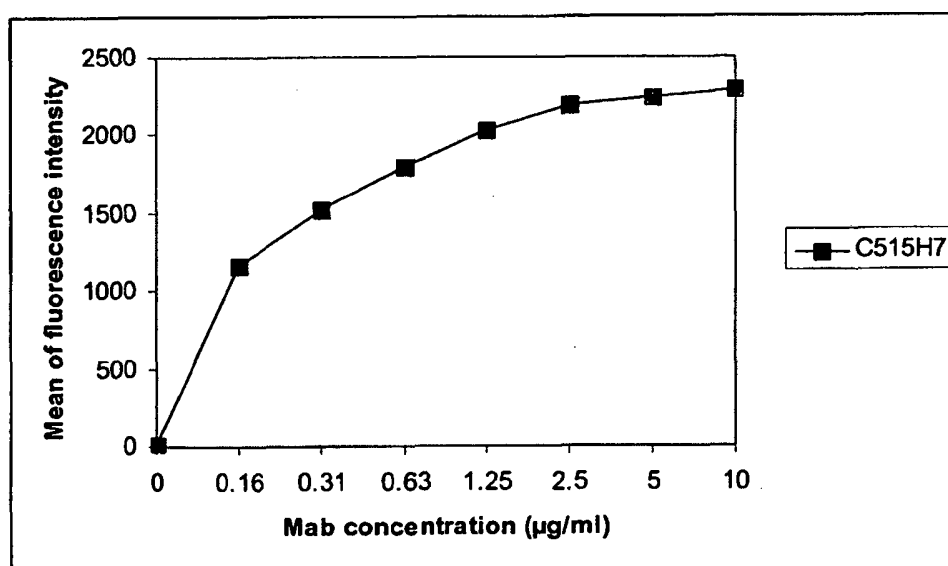


Figure 15

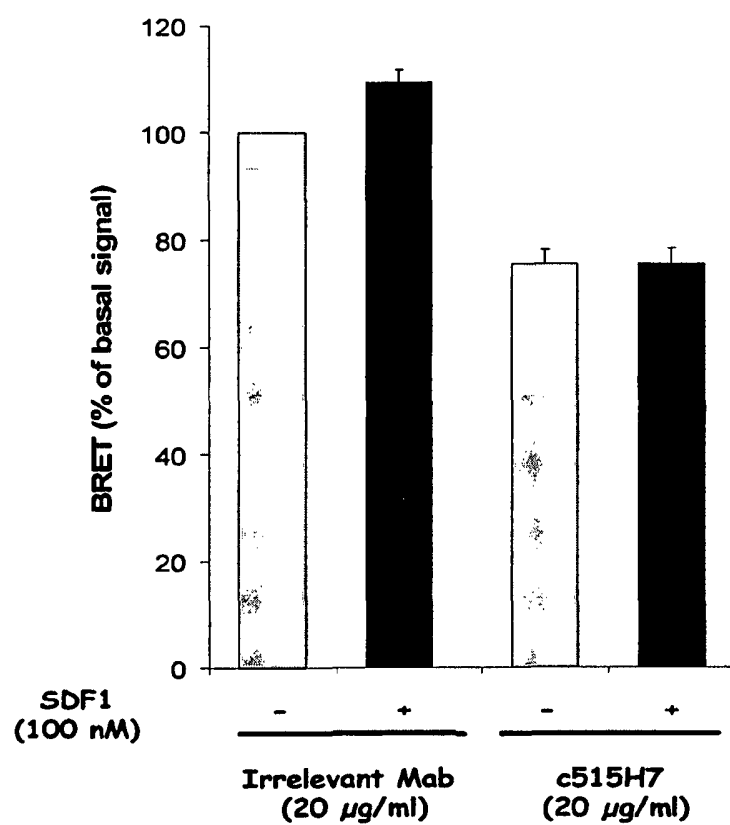


Figure 16

	FR1-IMGT (1-26)			CDR1-IMGT (27-38)		FR2-IMGT (39-55)		CDR2-IMGT (56-65)	
	1	10	20	30		40	50	60	
515H7 VH		EVNLVESGG	GLVQPGGSLRL	SCATS	GFTF...TDNY	MSWVRQPPGKALEW	LGF	IRNKANGYTT	
IGHV3-49*04		EVQLVESGG	GLVQPGRSLRL	SCTAS	GFTF...GDYA	MSWVRQAPGKGLEW	VGF	IRSKAYGGTT	
VH1		EVQLVESGG	GLVQPGRSLRL	SCTAS	GFTF...TDNY	MSWVRQAPGKGLEW	VGF	IRNKANGYTT	
VH1 D76N		EVQLVESGG	GLVQPGRSLRL	SCTAS	GFTF...TDNY	MSWVRQAPGKGLEW	VGF	IRNKANGYTT	
			FR3-IMGT (66-104)				CDR3-IMGT (105-115)		FR4-IMGT (116-125)
	70	80	90	100					
515H7 VH		DYSASVR	GRFTISRDN	SQSILYLQ	MNALRAED	SATYYC	ARDVGSNYFDYW	GQGTTLTVSS	
IGHV3-49*04		EYAASVK	GRFTISRDD	SKSIAYLQ	MNSLKTED	TAVYYC	TR		
IGHJ4*01							YFDYW	GQGTTLTVSS	
VH1		EYAASVK	GRFTISRDD	SKSIAYLQ	MNSLKTED	TAVYYC	ARDVGSNYFDYW	GQGTTLTVSS	
VH1 D76N		EYAASVK	GRFTISRDN	SKSIAYLQ	MNSLKTED	TAVYYC	ARDVGSNYFDYW	GQGTTLTVSS	

Figure 17

	FR1-IMGT (1-26)			CDR1-IMGT (27-38)		FR2-IMGT (39-55)		CDR2-IMGT (56-65)	
	1	10	20	30	40	50	60		
515H7 VL								WA.....	S
IGKV4-1*01	DIVMSQSPSSLAVSAGEKVTMSCKSS	QSLFNSRTRKNY	LAWYQQKPGQSPKLLIY	WA.....	S				
VL Var2	DIVMTQSPDSLAVSLGERATINCKSS	QSVLYSSNNKNY	LAWYQQKPGQPPKLLIY	WA.....	S				
VL Var2.1	DIVMTQSPSSLAVSLGERATMSCKSS	QSLFNSRTRKNY	LAWYQQKPGQSPKLLIY	WA.....	S				
VL Var2.2	DIVMTQSPDSLAVSLGERATMSCKSS	QSLFNSRTRKNY	LAWYQQKPGQPPKLLIY	WA	S				
VL Var2.3	DIVMTQSPDSLAVSLGERATMSCKSS	QSLFNSRTRKNY	LAWYQQKPGQPPKLLIY	WA	S				
	FR3-IMGT (66-104)			CDR3-IMGT (106-113)		FR4-IMGT (114-123)			
	70	80	90	100					
515H7 VL									
IGKV4-1*01	ARDSGVP.ARFTGSG..SETYFTLTISRVAEDLAVYYC	MQSFNLRT	FGQGTKVEIK						
IGKJ1*01	TRESGVP.DRFSGSG..SGTDFTLTISSLQAEDEVAVYYC								
VL Var2	ARDSGVP.ARFTGSG..SETYFTLTISRVAEDLAVYYC	MQSFNLRT	FGQGTKVEIK						
VL Var2.1	ARDSGVP.DRFSGSG.SETYFTLTISRVAEDLAVYYC	MQSFNLRT	FGQGTKVEIK						
VL Var2.2	ARDSGVP.DRFTGSG.SETYFTLTISRVAEDLAVYYC	MQSFNLRT	FGQGTKVEIK						
VL Var2.3	ARDSGVP.DRFTGSG.SETYFTLTISSLQAEDEVAVYYC	MQSFNLRT	FGQGTKVEIK						

Figure 18

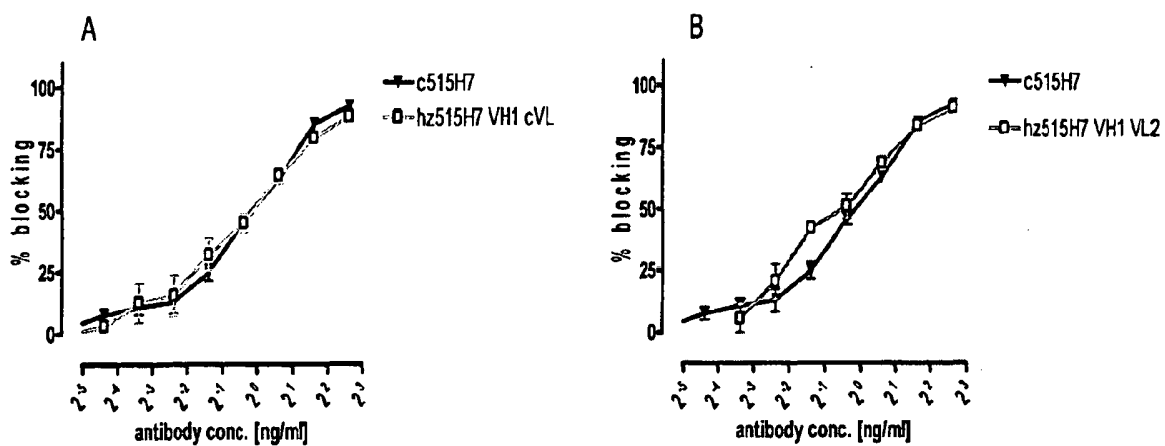


Figure 19

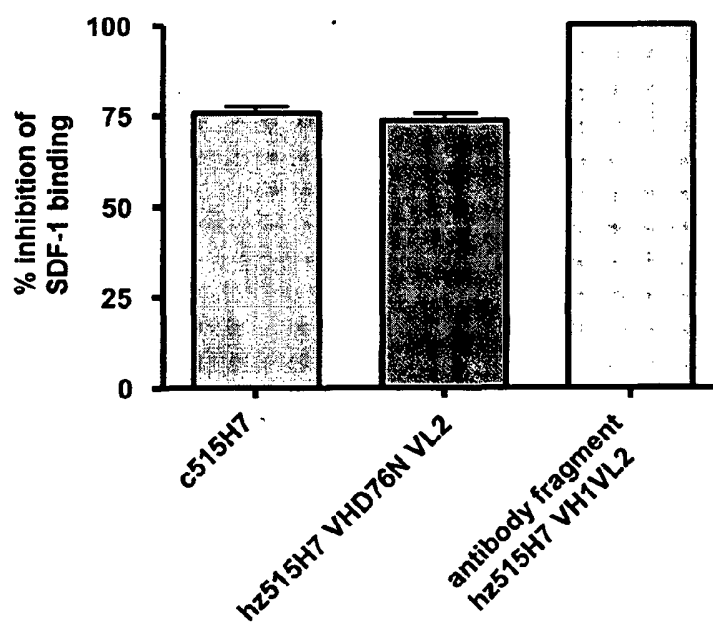


Figure 20

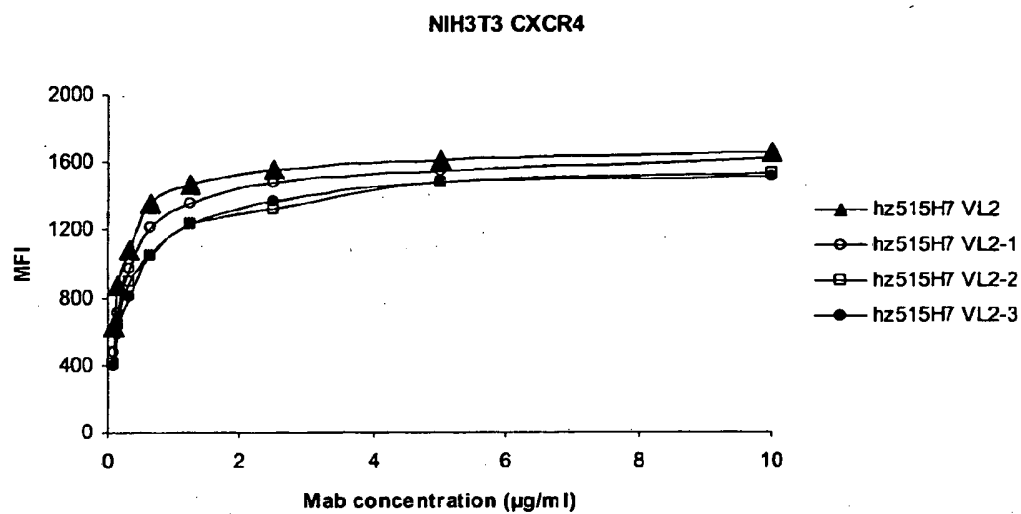


Figure 21

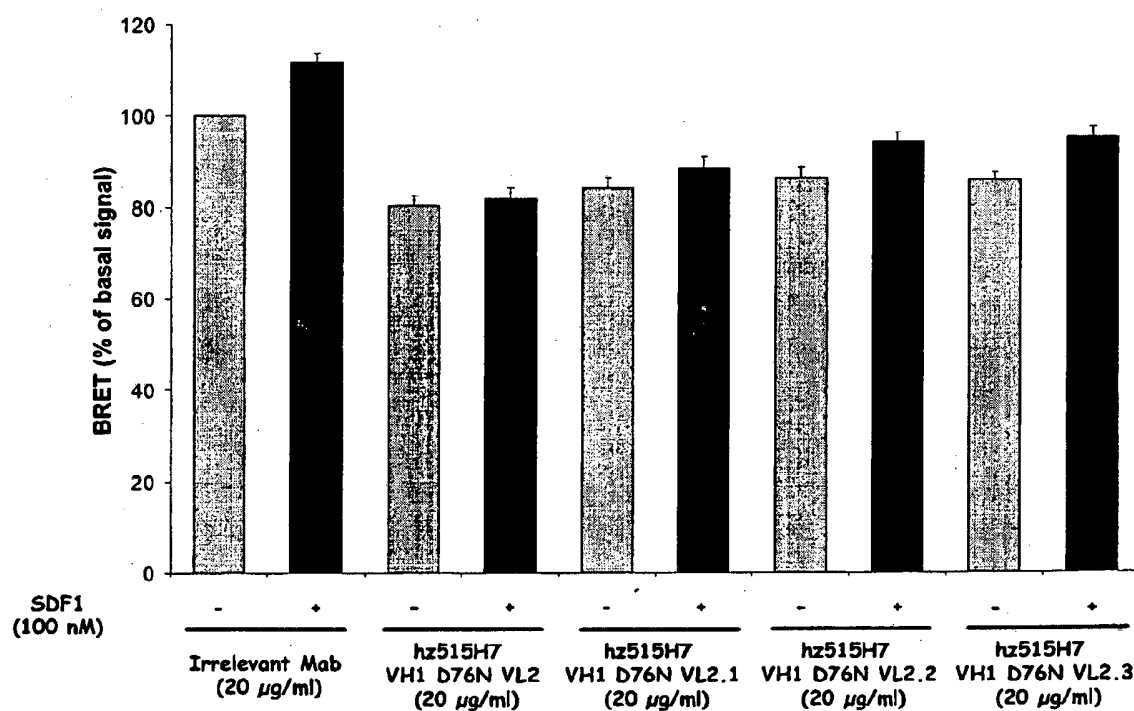


Figure 22

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- EP 0451261 A [0119]
- EP 0682040 A [0119]
- EP 0939127 A [0119]
- EP 0566647 A [0119]
- US 5530101 A [0119]
- US 6180370 B [0119]
- US 5585089 A [0119]
- US 5693761 A [0119]
- US 5639641 A [0119]
- US 6054297 A [0119]
- US 5886152 A [0119]
- US 5877293 A [0119]
- EP 09159076 A [0273]
- US 61173680 B [0273]

Non-patent literature cited in the description

- KOHLER ; MILSTEIN. *Nature*, 1975, vol. 256, 495-497 [0016]
- HOLL et al. *J. Immunol.*, 2004, vol. 173, 6274-83 [0019]
- ANGERS et al. *PNAS*, 2000, vol. 97, 3684-89 [0021]
- TANAKA R. et al. *J. Virol.*, 2001, vol. 75, 11534-11543 [0027]
- KABAT et al. Sequences of proteins of immunological interest. NIH, 1991 [0059]
- LEFRANC M.-P. *Immunology Today*, 1997, vol. 18, 509 [0061]
- LEFRANC M.-P. *The Immunologist*, 1999, vol. 7, 132-136 [0061]
- LEFRANC, M.-P. ; POMMIÉ, C. ; RUIZ, M. ; GIUDICELLI, V. ; FOULQUIER, E. ; TRUONG, L. ; THOUVENIN-CONTET, V. ; LEFRANC. *Dev. Comp. Immunol.*, 2003, vol. 27, 55-77 [0061]
- RUIZ, M. ; LEFRANC, M.-P. *Immunogenetics*, 2002, vol. 53, 857-883 [0061]
- KAAS, Q. ; LEFRANC, M.-P. *Current Bioinformatics*, 2007, vol. 2, 21-30 [0061]
- KAAS, Q. ; RUIZ, M. ; LEFRANC, M.-P. T cell receptor and MHC structural data. *Nucl. Acids. Res.*, 2004, vol. 32, D208-D210 [0061]
- SMITH ; WATERMAN. *Ad. App. Math.*, 1981, vol. 2, 482 [0067]
- NEDDLEMAN ; WUNSCH. *J. Mol. Biol.*, 1970, vol. 48, 443 [0067]
- PEARSON ; LIPMAN. *Proc. Natl. Acad. Sci. USA*, 1988, vol. 85, 2444 [0067]
- TATUSOVA et al. Blast 2 sequences - a new tool for comparing protein and nucleotide sequences. *FEMS Microbiol.*, 1999, *Lett.*, 1999, vol. 174, 247-250, <http://www.ncbi.nlm.nih.gov/gorf/bl2.html> [0069]
- SKERRA A. *J. Mol. Recogn.*, 2000, vol. 13, 167-187 [0076]
- SKERRA A. *J. Biotechnol.*, 2001, vol. 74 (4), 257-75 [0077]
- KOHL et al. *PNAS*, 2003, vol. 100 (4), 1700-1705 [0077] [0086]
- BES et al. *Biochem. Biophys. Res. Commun.*, 2006, vol. 343 (1), 334-344 [0079]
- NICAISE et al. *Protein Science*, 2004, vol. 13 (7), 1882-1891 [0079]
- BES et al. *FEBS letters*, 2001, vol. 508, 67-74 [0080]
- VERHOEYEN et al. *BioEssays*, 1988, vol. 8, 74 [0105]
- ANGAL S ; KING DJ ; BODMER MW ; TURNER A ; LAWSON AD ; ROBERTS G ; PEDLEY B ; ADAIR JR. A single amino acid substitution abolishes the heterogeneity of chimeric mouse/human (IgG4) antibody. *Mol Immunol.*, 1993, vol. 30 (1), 105-108 [0109] [0145]
- JONES et al. *Nature*, 1986, vol. 321, 522-525 [0118]
- VERHOEYEN et al. *Science*, 1988, vol. 239, 1534-1536 [0118]
- RIECHMANN et al. *Nature*, 1988, vol. 332, 323-327 [0118]
- SINGER et al. *J. Immun.*, 1992, vol. 150, 2844-2857 [0119]
- MOUNTAIN et al. *Biotechnol. Genet. Eng. Rev.*, 1992, vol. 10, 1-142 [0119]
- BEBBINGTON et al. *BiolTechnology*, 1992, vol. 10, 169-175 [0119]
- SAMBROOK et al. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, 2001 [0154]
- STEWARD et al. Solid phase peptides synthesis. Pierce Chem. Company, 1984 [0170]
- HOLLINGER ; BOHLEN. *Cancer and metastasis, rev.*, 1999, vol. 18, 411-419 [0175]
- GLENNIE MJ et al. *J. Immunol.*, 1987, vol. 139, 2367-2375 [0175]
- REPP R. et al. *J. Hemat.*, 1995, 377-382 [0175]
- STAERZ U.D. ; BEVAN M.J. *PNAS*, 1986, vol. 83, 1453-1457 [0175]
- SURESH M.R. et al. *Method Enzymol.*, 1986, vol. 121, 210-228 [0175]

EP 2 424 897 B1

- **MERCHAND et al.** *Nature Biotech.*, 1998, vol. 16, 677-681 [0175]
- **PARK et al.** *Mol. Immunol.*, 2000, vol. 37 (18), 1123-30 [0176]
- **ZHAO Q. et al.** *AIDS Research And Human Retroviruses*, 2003, vol. 19, 947-955 [0212]
- **JUAREZ J. et al.** *Leukemia*, 2003, vol. 17, 1294-1300 [0212]

ANTI-CXCR4 ELLENANYAGOK HIV KEZELÉSÉRE

Szabadalmi igénypontok

1. Izolált anti-CXCR4 ellenanyag vagy működőképes fragmense, amely működőképes fragmens az Fv, Fab, (Fab')₂, Fab', scFv, scFv-Fc fragmensek és diatestek közül választott, *azzal jellemezve*, hogy tartalmaz
 - rendre a SEQ ID No. 1, 2 és 3 szekvencia szerinti aminosav-szekvenciákat tartalmazó CDR-L1-et, CDR-L2-öt és CDR-L3-at tartalmazó könnyűláncot, és rendre a SEQ ID No. 4, 5 és 6 szekvencia szerinti aminosav-szekvenciákat tartalmazó CDR-H1-et, CDR-H2-öt és CDR-H3-at tartalmazó nehézláncot, vagy
 - rendre a SEQ ID No. 1, 2 és 30 szekvencia szerinti aminosav-szekvenciákat tartalmazó CDR-L1-et, CDR-L2-öt és CDR-L3-at tartalmazó könnyűláncot és rendre a SEQ ID No. 31, 32 és 33 szekvencia szerinti aminosav-szekvenciákat tartalmazó CDR-H1-et, CDR-H2-öt és CDR-H3-at tartalmazó nehézláncot.
2. Az 1. igénypont szerinti ellenanyag vagy működőképes fragmense, *azzal jellemezve*, hogy az ellenanyag
 - SEQ ID No. 7 szekvencia szerinti aminosavat tartalmazó könnyűláncot és SEQ ID No. 8 szekvencia szerinti aminosavat tartalmazó nehézláncot tartalmazó ellenanyag, vagy
 - SEQ ID No. 34 szekvencia szerinti aminosavat tartalmazó könnyűláncot és SEQ ID No. 35 szekvencia szerinti aminosavat tartalmazó nehézláncot tartalmazó ellenanyag.
3. Az 1. igénypont szerinti ellenanyag vagy működőképes fragmense, *azzal jellemezve*, hogy kiméra ellenanyag, és a SEQ ID No. 56, 57 vagy 58 szekvenciák alkotta csoportból választott nehézlánc-szekvenciát és SEQ ID No. 59 szerinti könnyűlánc-szekvenciát tartalmaz.
4. Az 1. igénypont szerinti ellenanyag, *azzal jellemezve*, hogy humanizált ellenanyag, és SEQ ID No. 64 szekvencia alkotta szekvenciájú nehézlánc variábilis régiót és SEQ ID No. 65, 66, 82 vagy 83 szekvenciák alkotta csoportból választott szekvenciájú könnyűlánc variábilis régiót tartalmaz.
5. Az 1. igénypont szerinti ellenanyag, *azzal jellemezve*, hogy humanizált ellenanyag, és a SEQ ID No. 67, 68 vagy 69 szekvenciák alkotta csoportból választott nehézlánc-szekvenciát és a SEQ ID No. 70, 71, 84 vagy 85 szekvenciák alkotta csoportból választott könnyűlánc-szekvenciát tartalmazó humanizált ellenanyagot vagy működőképes fragmensét tartalmazza.
6. Az 1. igénypont szerinti ellenanyag, *azzal jellemezve*, hogy a működőképes fragmenst scFv fragmens alkotja, amely scFv fragmens tartalmazza a SEQ ID No. 54 szerinti aminosav-szekvenciát.
7. Izolált nukleinsav, *azzal jellemezve*, hogy 1-6. igénypontok bármelyike szerinti ellenanyagot vagy működőképes fragmensét kódoló nukleinsav, DNS vagy RNS.
8. Vektor, amely 7. igénypont szerinti nukleinsavat tartalmaz.
9. Gazdasejt, amely 8. igénypont szerinti vektort tartalmaz.

10. Eljárás 1-6. igénypontok bármelyike szerinti ellenanyag vagy egy funkcionális fragmensének előállítására, *azzal jellemezve*, hogy magában foglalja a következő szakaszokat:

- a) 9. igénypont szerinti sejt tenyésztése tenyésztőközegben és megfelelő tenyésztési körülmények között, és
- b) az így módon előállított ellenanyag vagy egy funkcionális fragmensének kinyerése a tenyésztőközegből vagy a tenyésztett sejtekből.

11. Az 1-6. igénypontok bármelyike szerinti ellenanyag gyógyszerként történő alkalmazásra.

12. Az 1-6. igénypontok bármelyike szerinti ellenanyag vagy működőképes fragmense, *azzal jellemezve*, hogy HIV-1 KON primer izolátum replikációját PBMC-ben legalább 5 µg/ml, előnyösen legalább 10 µg/ml IC₅₀-értékkel gátolja.

13. Gyógyászati készítmény, amely hatóanyagként 1-6. igénypontok bármelyike szerinti vagy 12. igénypont szerinti ellenanyag vagy működőképes fragmense alkotja vagyületet tartalmaz.

14. A 13. igénypont szerinti készítmény HIV-fertőzés megelőzésére vagy kezelésére.

15. A 14. igénypont szerinti készítmény, *azzal jellemezve*, hogy a HIV-fertőzés X4-tropikus HIV-fertőzés és/vagy X4/R5-tropikus HIV-fertőzés.

16. A 13-15. igénypontok bármelyike szerinti készítmény, *azzal jellemezve*, hogy tartalmaz legalább második, a következők alkotta csoportból választott, HIV-elleni vegyületet: antiretrovirális gyógyszerek, például HIV-proteáz-inhibitorok (PI), nukleozid/nukleotid HIV-reverz-transzkriptáz inhibitorok (NRTI/NiRTI), nem nukleozid HIV-reverz-transzkriptáz inhibitorok (NNRTI), HIV-belépési gátlók, HIV-integráz inhibitorok.

17. A 15. vagy 16. igénypont szerinti készítmény, *azzal jellemezve*, hogy a legalább egy HIV-elleni szer a Maraviroc.

18. Eljárás molekulák szűrésére és CXCR4-antagonista antivirális szerként történő azonosítására, amely magában foglalja a következő lépéseket:

- a) CXCR4-et expresszáló sejtek kiválasztása,
- b) a sejtek inkubálása 1-6. igénypontok bármelyike szerinti vagy 12. igénypont szerinti ellenanyaggal vagy működőképes fragmensével vagy származékával, és
- c) a tesztelt molekulák értékelése az ellenanyag vagy egy működőképes fragmense vagy származéka és CXCR4 közötti kötés lehetséges gátlása alapján, és
- d) a gátlásra képes molekulák kiválasztása.