ANTI CXCR4 ANTIBODIES FOR THE TREATMENT OF HIV

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, functional fragments or derivatives, 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, functional fragments or derivatives as a medicament for the preventive and/or therapeutic treatment of HIV infection.

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

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.

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 (CELSENTRI®), 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.

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

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

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.

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.

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.

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.

In one aspect, there is provided 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.

In another aspect, there is provided 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 defined herein.

In another aspect, there is provided a vector comprising a nucleic acid as defined herein.

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

In another aspect, there is provided a process for production of an antibody, or one of its functional fragments, as defined herein characterized in that it comprises the following stages: a) culturing in a medium and appropriate culture conditions of a cell as defined herein; and b) the recovery of said antibodies, or one of their functional fragments, thus produced starting from the culture medium or said cultured cells.

In another aspect, there is provided the antibody as defined herein as a medicament.

In another aspect, there is provided a pharmaceutical composition comprising by way of active principle a compound consisting of an antibody, or one of its functional fragments, as defined herein.

In another aspect, there is provided the use of the composition as defined herein in the manufacture of a medicament for the prevention or for the treatment of HIV infection.

In another aspect, there is provided 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, as defined herein, 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.

In another aspect, there is provided the composition as defined herein for use in therapy.

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:

MEGISIYTSDNYTEEMGSGDYDSMKEPCFREENANFNKIFLPTIYSIIFLTGIVGN
GLVILVMGYQKKLRSMTDKYRLHLSVADLLFVITLPFWAVDAVANWYFGNFL
CKAVHVIYTVNLYSSVLILAFISLDRYLAIVHATNSQRPRKLLAEKVVYVGVWI
PALLLTIPDFIFANVSEADDRYICDRFYPNDLWVVVFQFQHIMVGLILPGIVILSC
YCIIISKLSHSKGHQKRKALKTTVILILAFFACWLPYYIGISIDSFILLEIIKQGCEFE
NTVHKWISITEALAFFHCCLNPILYAFLGAKFKTSAQHALTSVSRGSSLKILSKG
KRGGHSSVSTESESSSFHSS;

- the chemokine (C-X-C motif) receptor 4 isoform a [Homo sapiens] having the sequence as depicted under the Genbank accession number NP_001008540 SEQ ID No. 28:

MSIPLPLLQIYTSDNYTEEMGSGDYDSMKEPCFREENANFNKIFLPTIYSIIFLTGI
VGNGLVILVMGYQKKLRSMTDKYRLHLSVADLLFVITLPFWAVDAVANWYFG
NFLCKAVHVIYTVNLYSSVLILAFISLDRYLAIVHATNSQRPRKLLAEKVVYVG
VWIPALLLTIPDFIFANVSEADDRYICDRFYPNDLWVVVFQFQHIMVGLILPGIVI
LSCYCIIISKLSHSKGHQKRKALKTTVILILAFFACWLPYYIGISIDSFILLEIIKQGC
EFENTVHKWISITEALAFFHCCLNPILYAFLGAKFKTSAQHALTSVSRGSSLKILS
KGKRGGHSSVSTESESSSFHSS;

- an alternate transcriptional splice variant or a natural variant thereof having at least 95 % identity with one of these b or a isoforms having the SEQ ID No. 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.

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:

MEDFNMESDSFEDFWKGEDLSNYSYSSTLPPFLLDAAPCEPESLEINKYFVVIIY
ALVFLLSLLGNSLVMLVILYSRVGRSVTDVYLLNLALADLLFALTLPIWAASKV
NGWIFGTFLCKVVSLLKEVNFYSGILLLACISVDRYLAIVHATRTLTQKRYLVKF

ICLSIWGLSLLLALPVLLFRRTVYSSNVSPACYEDMGNNTANWRMLLRILPQSF GFIVPLLIMLFCYGFTLRTLFKAHMGQKHRAMRVIFAVVLIFLLCWLPYNLVLL ADTLMRTQVIQETCERRNHIDRALDATEILGILHSCLNPLIYAFIGQKFRHGLLKI LAIHGLISKDSLPKDSRPSFVGSSSGHTSTTL;

- an alternate transcriptional splice variant or a natural variant thereof having at least 95 % identity with this interleukin 8 receptor beta having the SEQ ID No. 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.

The invention also comprises 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, a subject of the present invention is a process for the generation and the selection of antibodies according to the invention.

More particularly, the invention concerns a process for the selection of an anti CXCR4 antibody, or one of its functional fragments or derivatives, 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.

In another embodiment, the invention concerns a process for the selection of an anti CXCR4 antibody, or one of its functional fragments or derivatives, 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.

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.

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.

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.

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).

In a preferred embodiment of the step iii) of selection of the process of the invention, 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.

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

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

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

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

In a particular aspect of the process according to the invention, 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.

In a second aspect, a subject of the invention is isolated antibodies, or one of their functional fragments or derivatives, 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.

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.

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

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

More particularly, according to another aspect of the invention, 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.

According to a first aspect, the invention 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.

According to a second aspect, the invention 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.

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

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

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

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

"Monoclonal antibody" is understood to mean an antibody arising from a nearly homogeneous antibody population. More particularly, the individual antibodies of a population are identical except for a few possible naturally-occurring mutations which can be found in minimal proportions. In other words, a monoclonal antibody consists of a homogeneous antibody arising from the growth of a single cell clone (for example a hybridoma, a eukaryotic host cell transfected with a DNA molecule coding for the homogeneous antibody, a prokaryotic host cell transfected with a DNA molecule coding for the homogeneous antibody, etc.) and is generally characterized by heavy chains of

one and only one class and subclass, and light chains of only one type. Monoclonal antibodies are highly specific and are directed against a single antigen. In addition, in contrast with preparations of polyclonal antibodies which typically include various antibodies directed against various determinants, or epitopes, each monoclonal antibody is directed against a single epitope of the antigen.

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

According to another embodiment, the antibodies of the invention, or one of their derived compounds or functional fragments, are characterized in that they comprise a light chain comprising at least one of the three CDRs of the sequences SEQ ID Nos.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.

The antibody of the invention, 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.

In another embodiment, the antibody of the invention, 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.

According to a second preferred embodiment of the invention, the antibody, or its derived compounds or functional fragments, is characterized in that it comprises a light chain comprising at least one CDR chosen 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.

According to another embodiment, the antibodies of the invention, or one of their derived compounds or functional fragments, are characterized in that they comprise a light chain comprising at least one of the three CDRs of the sequences SEQ ID Nos.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.

The antibody of the invention, 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.

In another embodiment, the antibody of the invention, 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.

More particularly, the antibodies of the invention, or one of their derived compounds or functional fragments, are characterized in that they comprise a heavy chain comprising at least one 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.

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. 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.

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.

In another embodiment, the antibody of the invention, 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.

More particularly, the antibodies of the invention, or one of their derived compounds or functional fragments, are characterized in that they comprise a heavy chain comprising at least one 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.

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.

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.

In another embodiment, the antibody of the invention, 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.

The antibody of the invention, or one of its functional fragments or derivatives, 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.

At last, the antibody of the invention, or one of its functional fragments or derivatives, 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.

The antibody of the invention, or one of its functional fragments or derivatives, 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.

At last, the antibody of the invention, or one of its functional fragments or derivatives, 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.

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

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

In a first embodiment, complementarity-determining region, or CDR, means the hypervariable regions of the heavy and light chains of immunoglobulins as defined by Kabat *et al.* (Kabat *et al.*, Sequences of proteins of immunological interest, 5th Ed., U.S. Department of Health and Human Services, NIH, 1991, and later editions). There are three heavy-chain CDRs and three light-chain CDRs. Here, the terms "CDR" and "CDRs" are used to indicate, depending on the case, one or more, or even all, of the regions containing the majority of the amino acid residues responsible for the antibody's binding affinity for the antigen or epitope it recognizes.

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

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

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

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

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

More particularly, 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.

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.

In the sense of the present invention, the "percentage identity" between two sequences of nucleic acids or amino acids means the percentage of identical nucleotides or amino acid residues between the two sequences to be compared, obtained after optimal alignment, this percentage being purely statistical and the differences between the two sequences being distributed randomly along their length. The comparison of two nucleic acid or amino acid sequences is traditionally carried out by comparing the sequences after having optimally aligned them, said comparison being able to be conducted by segment or by using an "alignment window". Optimal alignment of the

sequences for comparison can be carried out, in addition to comparison by hand, by means of the local homology algorithm of Smith and Waterman (1981) [Ad. App. Math. 2:482], by means of the local homology algorithm of Neddleman and Wunsch (1970) [J. Mol. Biol. 48:443], by means of the similarity search method of Pearson and Lipman (1988) [Proc. Natl. Acad. Sci. USA 85:2444] or by means of computer software using these algorithms (GAP, BESTFIT, FASTA and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI, or by the comparison software BLAST NR or BLAST P).

The percentage identity between two nucleic acid or amino acid sequences is determined by comparing the two optimally-aligned sequences in which the nucleic acid or amino acid sequence to compare can have additions or deletions compared to the reference sequence for optimal alignment between the two sequences. Percentage identity is calculated by determining the number of positions at which the amino acid nucleotide or residue is identical between the two sequences, preferably between the two complete sequences, dividing the number of identical positions by the total number of positions in the alignment window and multiplying the result by 100 to obtain the percentage identity between the two sequences.

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

For the amino acid sequence exhibiting at least 80%, preferably 85%, 90%, 95% and 98% identity with a reference amino acid sequence, preferred examples include those containing the reference sequence, certain modifications, notably a deletion, addition or substitution of at least one amino acid, truncation or extension. In the case of substitution of one or more consecutive or non-consecutive amino acids, substitutions are preferred in which the substituted amino acids are replaced by "equivalent" amino acids. Here, the expression "equivalent amino acids" is meant to indicate any amino

acids likely to be substituted for one of the structural amino acids without however modifying the biological activities of the corresponding antibodies and of those specific examples defined below.

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

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

Table 1

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

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

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

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

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

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

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

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

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

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

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).

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

a) the placing in contact *in vitro* of a compound composed of a peptide scaffold on which is grafted at least one antibody CDR with a biological sample containing HIV type 1 and PBMC; and

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

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.

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

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

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

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

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

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

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

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

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

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

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

For more clarity, table 2 below summarizes the various amino acid sequences corresponding to the 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

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).

More particularly, as an example, the antibodies of the invention, or one of their functional fragments or derivatives, have no affinity for the FcγR (I, II or III) or for the C1q, or for both of them.

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

Consequence of this is that the antibodies of the invention are preferably selected form IgG4 or IgG2 isotypes, most preferably IgG4.

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.

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

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

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.

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.

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

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

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

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

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) .

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).

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].

More particularly, the invention relates to a chimeric antibody heavy chain characterized in that it 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.

More particularly, the invention relates to a chimeric antibody light chain characterized in that it 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.

More particularly, the invention relates to a chimeric antibody, or a derived compound or 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.

In another embodiment, the invention relates to a chimeric antibody, or a derived compound or 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.

In still another embodiment, the invention relates to a chimeric antibody, or a derived compound or 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.

In a preferred embodiment, the chimeric antibody c515H7 VH(G4wt) / VL-Ck, or a derived compound or 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.

In a preferred embodiment, the chimeric antibody c515H7 VH(G4PRO) / VL-Ck, or a derived compound or 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.

In a preferred embodiment, the chimeric antibody c515H7 VH(G2wt) / VL-Ck, or a derived compound or 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.

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

The humanized antibodies of the invention or fragments of same can be prepared by techniques known to a person skilled in the art (such as, for example, those described in the documents Singer et al., J. Immun., 150:2844-2857, 1992; Mountain et al., Biotechnol. Genet. Eng. Rev., 10:1-142, 1992; and Bebbington et al., Bio/Technology, 10:169-175, 1992). Such humanized antibodies are preferred for their use in methods involving in vitro diagnoses or preventive and/or therapeutic treatment in vivo. Other humanization techniques, also known to a person skilled in the art, such as, for example, the "CDR grafting" technique described by PDL in patents EP 0 451 216, EP 0 682 040, EP 0 939 127, EP 0 566 647 or US 5,530,101, US 6,180,370, US 5,585,089 and US 5,693,761. US patents 5,639,641 or 6,054,297, 5,886,152 and 5,877,293 can also be cited.

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

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

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.

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

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.

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.

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.

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.

More particularly, the invention relates to a humanized antibody, or a derived compound or 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.

In another embodiment, the invention relates to a humanized antibody, or a derived compound or 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.

In still another embodiment, the invention relates to a humanized antibody, or a derived compound or 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.

In a preferred embodiment, the humanized antibody Hz515H7 VH1 D76N (G4wt) / VL2-Ck, or a derived compound or 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.

In another preferred embodiment, the humanized antibody Hz515H7 VH1 D76N (G4PRO) / VL2-Ck, or a derived compound or 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.

In another preferred embodiment, the humanized antibody Hz515H7 VH1 D76N (G2wt) / VL2-Ck, or a derived compound or 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.

In another preferred embodiment, the humanized antibody Hz515H7 VH1 D76N (G4wt) / VL2.1-Ck, or a derived compound or 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.

In another preferred embodiment, the humanized antibody Hz515H7 VH1 D76N (G4PRO) / VL2.1-Ck, or a derived compound or 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.

In another preferred embodiment, the humanized antibody Hz515H7 VH1 D76N (G2wt) / VL2.1-Ck, or a derived compound or 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.

In another preferred embodiment, the humanized antibody Hz515H7 VH1 D76N (G4wt) / VL2.2-Ck, or a derived compound or 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.

In another preferred embodiment, the humanized antibody Hz515H7 VH1 D76N (G4PRO) / VL2.2-Ck, or a derived compound or 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.

In another preferred embodiment, the humanized antibody Hz515H7 VH1 D76N (G2wt) / VL2.2-Ck, or a derived compound or 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.

In another preferred embodiment, the humanized antibody Hz515H7 VH1 D76N (G4wt) / VL2.3-Ck, or a derived compound or 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.

In another preferred embodiment, the humanized antibody Hz515H7 VH1 D76N (G4PRO) / VL2.3-Ck, or a derived compound or 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.

In another preferred embodiment, the humanized antibody Hz515H7 VH1 D76N (G2wt) / VL2.3-Ck, or a derived compound or 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.

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

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*).

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].

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).

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..

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 or derivatives, 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.

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
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

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

It should also be included here that the present invention does not relate to nucleotide sequences in their natural chromosomal environment, i.e., in a natural state. The sequences of the present invention have been isolated and/or purified, i.e., they

were sampled directly or indirectly, for example by a copy, their environment having been at least partially modified. Isolated nucleic acids obtained by recombinant genetics, by means, for example, of host cells, or obtained by chemical synthesis should also be mentioned here.

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

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

DNA-DNA or DNA-RNA hybridization is carried out in two steps: (1) prehybridization at 42°C for three hours in phosphate buffer (20 mM, pH 7.5) containing 5X SSC (1X SSC corresponds to a solution of 0.15 M NaCl + 0.015 M sodium citrate), 50% formamide, 7% sodium dodecyl sulfate (SDS), 10X Denhardt's, 5% dextran sulfate and 1% salmon sperm DNA; (2) primary hybridization for 20 hours at a temperature depending on the length of the probe (i.e.: 42°C for a probe >100 nucleotides in length) followed by two 20-minute washings at 20°C in 2X SSC + 2% SDS, one 20–minute washing at 20°C in 0.1X SSC + 0.1% SDS. The last washing is carried out in 0.1X SSC + 0.1% SDS for 30 minutes at 60°C for a probe >100 nucleotides in length. The highly stringent hybridization conditions described above for a polynucleotide of defined size can be adapted by a person skilled in the art for longer or shorter oligonucleotides, according to the procedures described in Sambrook, *et al.*

(Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory; 3rd edition, 2001).

The invention also 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;

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;

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.

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

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

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 or derivatives, 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.

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

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

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

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

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

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

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

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

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

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

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

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

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

The polypeptides of the invention can also be prepared by chemical synthesis. One such method of preparation is also an object of the invention. A person skilled in the art knows methods for chemical synthesis, such as solid-phase techniques (see

notably Steward *et al.*, 1984, Solid phase peptides synthesis, Pierce Chem. Company, Rockford, 111, 2nd ed.) or partial solid-phase techniques, by condensation of fragments or by conventional synthesis in solution. Polypeptides obtained by chemical synthesis and capable of containing corresponding unnatural amino acids are also comprised in the invention.

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

According to still another aspect, the present invention relates to 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.

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.

According to a novel embodiment, the invention 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.

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).

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

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

In a preferred embodiment of the invention, the bispecific antibodies are bivalent or tetravalent antibodies.

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

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.

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

In a particular aspect of the invention, the antibody, or one of its functional fragments or derivatives, inhibits HIV-1 KON primary isolate replication in PBMC with an IC_{90} of at least 5 µg/ml, preferably at least 10 µg/ml.

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.

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

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

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.

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

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

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.

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 that an antibody directed against CXCR4.

According to still another embodiment, the present invention also relates to a pharmaceutical composition as described above that comprises at least a second 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.

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.

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

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

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

Generally, the composition according to the invention considerably increases 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.

"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".

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.

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®)

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/atripla/truvada®), Emtricitabine or FTC (Emtriva/atripla/truvada®)

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/atripla®, Stocrin®), Delavirdine or DLV (Rescriptor®) and Etravirine or ETR (Intelence®).

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®).

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®).

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.

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 ...).

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.

In order to facilitate binding between said anti-HIV agent and the antibody according to the invention, spacer molecules can be introduced between the two compounds to bind, such as the poly(alkylene)glycol polyethyleneglycol or the amino acids; or, in another embodiment, said 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.

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

The 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.

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

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

As previously demonstrated, CXCR4 Mabs 515H7 and 301aE5 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).

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 or derivatives, 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 or derivatives, to CXCR4, and

d) selecting molecules capable of said inhibition.

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

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

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

FIGURE LEGENDS

Figures 1A and 1B show 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

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 bellow) and mice with the higher titers of anti-CXCR4 antibodies were used for fusions. Mice were boost intravenously with antigen two days before sacrifice and removal of the spleen.

- *ELISA*

To select the mice producing anti-CXCR4 antibodies, sera from immunized mice was tested by ELISA. Briefly, microtiter plates were coated with purified [1-41] N-terminal peptide conjugated to BSA at 5µg equivalent peptide/mL, 100µL/well incubated at 4°C overnight, then blocked with 250µL/well of 0.5% gelatine in PBS. Dilutions of plasma from CXCR4-immunized mice were added to each well and incubated 2 hours at 37°C. The plates were washed with PBS and then incubated with a goat anti-mouse IgG antibody conjugated to HRP (Jackson Laboratories) for 1 hour at 37°C. After washing, plates were developed with TMB substrate, the reaction was stopped 5 min later by addition of 100 µL/well 1M H_2SO_4. Mice that developed the highest titers of anti-CXCR4 antibodies were used for antibody generation.

- *Generation of hybridomas producing Mabs to CXCR4*

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

Example 2: Characterization of anti-CXCR4 Mab 515H7 and 301aE5 binding specificity (NIH3T3-CXCR4 transfectant) by FACS analysis

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-*h*CXCR4 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

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).

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

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.

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 CO_2 5 % in order to allow cell attachment to the plate. Cells were then starved with 200 µl DMEM/well overnight. Immediately prior to the BRET experiment, DMEM was removed and cells were quickly washed with PBS. Cells were then

incubated in PBS in the presence or absence of antibody, 10 min at 37°C prior to the addition of coelenterazine H 5 µM with or without SDF-1 300 nM in a final volume of 50 µl. After incubation for further 10 minutes at 37°C, light-emission acquisition at 485 nm and 530 nm was initiated using the Mithras LB940 multilabel reader (Berthold) (1s/wavelength/well repeated 15 times at room temperature).

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

SDF1 (300 nM) increased by about 20 % the BRET signal resulting from the spatial proximity of the adaptor and acceptor proteins fused to CXCR4 receptor, it is likely to indicate CXCR4/CXCR4 homo-dimers formation or conformational changes of pre-existing dimers (Figures 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

PBMC from normal donors seronegative for HIV-1 were isolated from buffy coats or cytapheresis 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 x 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 Cytofix/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

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

CHO-K1 cells, stably and constitutively expressing human CXCR4 receptor were obtained upon transfection of naïve CHO-K1 cells (ATCC CCL-61) with a mammalian expression vector carrying the whole coding sequence of human CXCR4 receptor (RefSeq NM_003467). Cells were propagated in complete culture medium [DMEM-Ham's F12 supplemented with 5 % fetal calf serum (FCS) and 500 µg/ml of geneticin]. 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).

SDF1 (100 nM) induced a rapid and strong release of intracellular calcium in recombinant CHO/CXCR4, whereas no fluorescence signal was detected in naïve CHO-K1 cells. The maximal intensity reached > 140 % over basal fluorescence and was observed at about 40 sec. upon stimulation by SDF-1 (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.

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.

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 10^6 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).

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/Immunotech, 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.

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.

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

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.

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 10^6 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).

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/Immunotech, 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.

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.

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 µg/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:

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 x 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 [$TCID_{50}$]) 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 ($V_n/V_0 = 0.1$).

As shown in Figure 12, the dual tropic X4R5 virus 89.6 replication was inhibited by c515H7 Mabs with an IC_{90} of 2µg/ml (Figure 12). Maraviroc at 50µg/ml did not reach the IC_{90} inhibitory activity (Figure 12). Moreover, addition of Maraviroc at 2µg/ml to antibody 515H7 augmented the inhibitory activity of the c515H7 Mabs with an IC_{90} of 0.2µg/ml (Figure 12).

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 µg/ml of each) allowed a 90 % reduction of virus titer (Figure 13).

Example 10: Production of anti-CXCR4 chimeric Mab c515H7

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).

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.

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).

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 2.10^6 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 µg/ml for heavy to light chain plasmid ratio of 1:1). At 4 hours post-transfection, the culture was diluted with one volume of fresh culture medium to achieve a final cell density of 10^6cells/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

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

NIH3T3-hCXCR4 transfected cells were incubated with a dose range of monoclonal antibody c515H7 from 0 µg/ml to 10 µg/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

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.

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 CO_2 5 % in order to allow cell attachment to the plate. Cells were then starved with 200 µl DMEM/well overnight. Immediately prior to the BRET experiment, DMEM was removed and cells were quickly washed with PBS. Cells were then incubated in PBS in the presence or absence of antibody, 10 min at 37°C prior to the addition of coelenterazine H 5 µM with or without SDF-1 100 nM in a final volume of 50 µl. After incubation for further 10 minutes at 37°C, light-emission acquisition at 485 nm and 530 nm was initiated using the Mithras LB940 multilabel reader (Berthold) (1s/wavelength/well repeated 15 times at room temperature).

Calculation of BRET ratio was performed as previously described (Angers et al., 2000): $[(\text{emission}_{530\ nm}) - (\text{emission}_{485\ nm})\ X\ Cf] / (\text{emission}_{485\ nm})$, where $Cf = (\text{emission}_{530\ nm}) / (\text{emission}_{485\ nm})$ for cells expressing the Rluc fusion protein alone under the same experimental conditions. Simplifying this equation shows that BRET

ratio corresponds to the ratio 530/485 nm obtained when the two BRET partners are present, corrected by the ratio 530/485 nm obtained under the same experimental conditions, when only the partner fused to Rluc is present in the assay. For sake of readability, results are expressed in milliBRET units (mBU); mBU corresponds to the BRET ratio multiplied by 1000.

SDF1 (100 nM) increased by about 10 % the BRET signal resulting from the spatial proximity of the donor and acceptor proteins fused to CXCR4 receptor, it is likely to indicate CXCR4/CXCR4 homo-dimers formation or conformational changes of pre-existing dimers (Figure 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.

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.

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 µl of Ghost cells were plated (2.5 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 µL 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.

The percent of inhibition of infected cells was defined as the compared with control infected wells with no Mab. Results of ICs (in µg/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.

	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

Table 11

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

- *General procedure*

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).

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.

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 IgG_1/κ-derived antibodies were transiently expressed in HEK293 cells. Expression culture supernatants were filtered and antibodies were purified using protein A sepharose. Purified antibodies were rebuffered in PBS and antibodies concentrations determined by ELISA.

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*

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

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).

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.

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

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

NIH3T3, NIH3T3-hCXCR4 transfected were incubated with 0 to 10 µg/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 µl 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.

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

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.

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 CO_2 5 % in order to allow cell attachment to the plate. Cells were then starved with 200 µl DMEM/well overnight. Immediately prior to the BRET experiment,

DMEM was removed and cells were quickly washed with PBS. Cells were then incubated in PBS in the presence or absence of antibody, 10 min at 37°C prior to the addition of coelenterazine H 5 µM with or without SDF-1 100 nM in a final volume of 50 µl. After incubation for further 10 minutes at 37°C, light-emission acquisition at 485 nm and 530 nm was initiated using the Mithras LB940 multilabel reader (Berthold) (1s/wavelength/well repeated 15 times at room temperature).

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

SDF1 (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).

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)

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 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 IC_{90} 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. Use of the composition as claimed in claim 13 in the manufacture of a medicament for the prevention or for the treatment of HIV infection.

15. The use 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 use according to claims 13 to 15, characterized in that the composition 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 use 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, 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, to CXCR4, and

d) selecting molecules capable of said inhibition.

19. The composition of claim 13 for use in therapy.