

## NOVEL ANTIPROLIFERATION ANTIBODIES

The present invention relates to novel antibodies, in particular murine monoclonal antibodies, chimeric and humanized, able to inhibit tumor growth, as well  
5 as the amino and nucleic acid sequences coding for such antibodies. From one aspect, the invention relates to novel antibodies, derived compounds or functional fragments, able to inhibit the proliferation of tumor cells. The invention also comprises the use of such antibodies as a drug for the preventive and/or therapeutic treatment of cancers, as well as in the procedures or kits related to cancer diagnosis. Finally, the invention  
10 comprises compositions comprising such antibodies in combination with other anticancer compounds, such as antibodies, or conjugated with toxins, and the use of same for the prevention and/or treatment of certain cancers.

Generally, the criterion chosen for the production of monoclonal antibodies is the recognition of the immunogen identified as a potential target of a treatment. In  
15 practice, mice are immunized with a recombinant protein that corresponds to the immunogen and, after the monoclonal antibodies produced by the mouse are recovered, they are first screened for their capacity to recognize the immunogen in a specific manner. In a second stage, the antibodies thus selected are tested *in vivo* and *in vitro* in order to determine their activity as well as their properties and/or mechanisms of action.

20 This "traditional" approach, even if it makes it possible to know the working target from the beginning, often generates a large number of antibodies which are certainly capable of specifically recognizing a given target but which *in vivo* do not exhibit significant biological activity. In the field of cancer, it is indeed known that, even if an antibody produces good results *in vitro*, that does not inevitably mean that  
25 such an antibody will later show genuine antitumor activity *in vivo*.

The present invention differs from this manner of proceeding, and goes even against the aforementioned, since it is based on a "functional" approach, and more particularly on primary screening based on the function sought for the antibody and not on the recognized antigen.

30 More particularly, the inventors have selected a given function, namely inhibition of basal proliferation, not induced, of the cell, as an antibody selection parameter.

The production method used will be described in more detail in the examples below.

In a surprising way, by this functional approach, the inventors have produced and selected an antibody capable of inhibiting *in vitro* and/or *in vivo*, in a significant manner, the proliferation of tumor cells.

According to one embodiment, the invention relates to an isolated antibody, or a derived compound or functional fragment of same, capable of inhibiting the proliferation of tumor cells *in vitro* and/or *in vivo*; said antibody, or a derived compound or functional fragment of same, comprising at least one CDR chosen among the complementarity-determining regions (CDRs) of sequences SEQ ID No. 1, 2, 3, 4, 5 or 6 or at least one CDR whose sequence has at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID No. 1, 2, 3, 4, 5 or 6.

A "functional fragment" of an antibody means in particular an antibody fragment, such as fragments Fv, scFv (sc=simple chain), Fab, F(ab')<sub>2</sub>, 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" 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 be recognized. 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  
5 contrast with preparations of polyclonal antibodies which typically include various antibodies directed against various determinants, or epitopes, each monoclonal antibody is directed against a single epitope of the antigen.

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

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

More particularly, the antibodies of the invention, or one of their derived compounds or functional fragments, are characterized in that they comprise a heavy chain comprising at least one of the three CDRs of the sequences SEQ ID Nos. 2, 4 and  
25 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. 2, 4 or 6.

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

- CDR-H1 comprises the sequence SEQ ID No. 2, 7 or 9, or a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 2, 7 or 9;

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

5       According to a particular embodiment, antibodies, or one of their derived compounds or functional fragments, are characterized in that they comprise a heavy chain comprising the CDR-H1 of the sequence SEQ ID No. 7, the CDR-H2 of the sequence SEQ ID No. 4 and the CDR-H3 of the sequence SEQ ID No. 12.

10       According to another particular embodiment, antibodies, or one of their derived compounds or functional fragments, are characterized in that they comprise a heavy chain comprising the CDR-H1 of the sequence SEQ ID No. 9, the CDR-H2 of the sequence SEQ ID No. 11 and the CDR-H3 of the sequence SEQ ID No. 6.

15       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, 3 and 5, 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, 3 or 5.

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

- CDR-L1 comprises the sequence SEQ ID No. 1 or 8, or a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 1 or 8;
- CDR-L2 comprises the sequences SEQ ID No. 3 or 10, or a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 3 or 10; and
- CDR-L3 comprises the sequence SEQ ID No. 5, or a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 5.

30       According to a particular embodiment, antibodies, or one of their derived compounds or functional fragments, are characterized in that they comprise a light chain comprising the CDR-L1 of the sequence SEQ ID No. 1, the CDR-L2 of the sequence SEQ ID No. 3 and the CDR-L3 of the sequence SEQ ID No. 5.

According to another particular embodiment, antibodies, or one of their derived compounds or functional fragments, are characterized in that they comprise a light chain comprising the CDR-L1 of the sequence SEQ ID No. 8, the CDR-L2 of the sequence SEQ ID No. 10 and the CDR-L3 of the sequence SEQ ID No. 5.

In a first aspect, there is provided an isolated antibody, or a derived compound or functional fragment of same, capable of inhibiting the proliferation of tumor cells *in vitro* and/or *in vivo*, characterized in that it comprises a light chain comprising the following three CDRs: CDR-L1 of the sequence SEQ ID No. 1; CDR-L2 of the sequence SEQ ID No.3; and CDR-L3 of the sequence SEQ ID No. 5; and a heavy chain comprising the following three CDRs: CDR-H1 of the sequence SEQ ID No. 2; CDR-H2 of the sequence SEQ ID No. 4; and CDR-H3 of the sequence SEQ ID No. 6.

In a second aspect, there is provided a murine hybridoma filed with the CNCM, Pasteur Institute, Paris, July 6, 2006, under number I-3646.

In a third aspect, there is provided an antibody secreted by the hybridoma according to the second aspect.

In a fourth aspect, there is provided an isolated nucleic acid characterized in that it is selected among the following nucleic acids: a) a nucleic acid, DNA or RNA, coding for an antibody, or for a derived compound or functional fragment of same, as defined above; b) a nucleic acid complementary to a nucleic acid as defined in a); c) a nucleic acid of at least 18 nucleotides capable of hybridizing under highly stringent conditions with at least one of the CDRs of nucleic acid sequences SEQ ID Nos. 20, 22 to 24, 26 and 27; and d) a nucleic acid of at least 18 nucleotides capable of hybridizing under highly stringent conditions with at least the light chain of nucleic acid sequence SEQ ID No. 32 or 36 and/or the heavy chain of nucleic acid sequence SEQ ID No. 33, 37 or 38.

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

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

In a seventh aspect, there is provided a method for producing an antibody, or a derived compound or functional fragment of same, as defined above, characterized in that said method comprises the following steps: a) the culture in a medium of and the suitable culture conditions for a host cell according to the sixth aspect; and b) the recovery of said antibody, or one of its functional fragments, thus produced from the culture medium or from said cultured cells.

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

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

In a tenth aspect, there is provided an antibody, or a derived compound or functional fragment of same, as defined above, or a composition as defined above, for the prevention or the treatment of a disease related to tumor or cell proliferation.

In an eleventh aspect, there is provided the use of an antibody, or a derived compound or functional fragment of same, as defined above, or a composition as defined above in the preparation of a medicament for the prevention or the treatment of a disease related to tumor cell proliferation.

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, 5<sup>th</sup> 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  
5 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  
10 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  
15 epitope which it recognizes.

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

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

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

More particularly, CDR-L1 consist of SEQ ID No. 1 (QDINNY) in the common  
25 and IMGT numbering systems and of SEQ ID No. 8 (KASQDINNYIA) in the kabat numbering system.

Concerning the CDR-L2, it consists of SEQ ID No. 3 (YTS) in the common and IMGT numbering systems and of SEQ ID No. 10 (YTSTLQA) in the kabat numbering system.

30 The CDR-L3 consists of SEQ ID No. 5 (LQYDNLWT) for each of the three numbering systems.

For the heavy chain, the CDR-H1 consists of the SEQ ID No. 2 (**TDYS**) in the common numbering system, of SEQ ID No. 7 (**GYSFTDYS**) in the IMGT numbering system and of SEQ ID No. 9 (**TDYSMY**) in the kabat numbering system.

The CDR-H2 consists of SEQ ID No. 4 (**IDPYNGGT**) in the common and  
5 IMGT numbering systems and of SEQ ID No. 11 (**YIDPYNGGTRYNQKFKG**) in the kabat numbering system.

At last, the CDR-H3 consists in the SEQ ID No. 6 (**QTDYFDY**) in the common and kabat numbering systems whereas it consists of SEQ ID No. 12 (**ARQTDYFDY**) in the IMGT numbering system.

10 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  
15 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.  
20 2:482], by means of the local homology algorithm of Needleman and Wunsch (1970) [J. Mol. Biol. 48:443], by means of the similarity search method of Pearson and Lipman (1988) [Proc. Natl. Acad. Sci. USA 85:2444] or by means of computer software using these algorithms (GAP, BESTFIT, FASTA and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI, or by the  
25 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  
30 identity is calculated by determining the number of positions at which the amino acid nucleotide or residue is identical between the two sequences, dividing the number of identical positions by the total number of positions in the alignment window and

multiplying the result by 100 to obtain the percentage identity between the two sequences.

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

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

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

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

Table 1

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

It is known by those skilled in the art that in the current state of the art the greatest variability (length and composition) between the six CDRs is found at the three heavy-chain CDRs and, more particularly, at CDR-H3 of this heavy chain. Consequently, it will be evident that the preferred characteristic CDRs of the antibodies of the invention, or of one of their derived compounds or functional fragments, will be the three CDRs of the heavy chain, i.e., the CDRs coded by sequences SEQ ID Nos. 2, 4 and 6, respectively, and even more preferentially, the CDR corresponding to the CDR-H3 coded by sequence SEQ ID No. 6.

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

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

- CDR-L1 of the sequence SEQ ID No. 1 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 1; CDR-L2 of the sequence SEQ ID No. 3 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 3;
- 5 and
- CDR-L3 of the sequence SEQ ID No. 5 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 5, and
- a heavy chain comprising the following three CDRs:
- 10 CDR-H1 of the sequence SEQ ID No. 7 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 7; CDR-H2 of the sequence SEQ ID No. 4 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 4; and
- 15 CDR-H3 of the sequence SEQ ID No. 12 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 12.
- Still another embodiment of the invention discloses an antibody, or a derived compound or functional fragment of same, comprising a light chain comprising the
- 20 following three CDRs:
- CDR-L1 of the sequence SEQ ID No. 8 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 8;
  - CDR-L2 of the sequence SEQ ID No. 10 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 10; and
  - 25 - CDR-L3 of the sequence SEQ ID No. 5 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 5, and
- a heavy chain comprising the following three CDRs:
- CDR-H1 of the sequence SEQ ID No. 9 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 9;
  - 30 - CDR-H2 of the sequence SEQ ID No. 11 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 11; and

- CDR-H3 of the sequence SEQ ID No. 6 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 6.

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

It is also disclosed a humanized antibody, or a derived compound or functional fragment of same, which is characterized in that it comprises a light chain sequence comprising the amino acid sequence SEQ ID No. 17 or a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 17, and in that it comprises a heavy chain sequence comprising the amino acid sequence SEQ ID No. 18 or 19 or a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 18 or 19.

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

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

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

Generally, a person skilled in the art knows how to determine the type of protein scaffold on which to graft at least one of the CDRs arising from the original antibody.

More particularly, it is known that to be selected such scaffolds must meet the greatest number of criteria as follows (Skerra A., J. Mol. Recogn., 2000, 13:167-187):

- good phylogenetic conservation;
- known three-dimensional structure (as, for example, by crystallography,  
5 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.

10 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-  
15 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.

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

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

- A specific aspect of the present invention relates to a method for selecting a compound derived from an antibody according to the invention, said derived compound being capable of inhibiting *in vitro* and/or *in vivo* the growth of tumor cells and said derived compound comprising a peptide scaffold on which is grafted at least one antibody CDR, characterized in that it comprises the following steps:
- 5     a)     the placing in contact *in vitro* of a compound composed of a peptide scaffold on which is grafted at least one antibody CDR with a biological sample containing tumor cells able to grow and under conditions allowing these cells to grow; and
- 10    b)     selection of said compound if said compound is capable of inhibiting the growth of these tumor cells,
- and characterized in that said at least one grafted CDR is selected among the following CDRs:
- 15       - the CDR of sequence SEQ ID No. 1, 8 or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 1, 8;
  - the CDR of sequence SEQ ID No. 3, 10 or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 3, 10;
  - 20       - the CDR of sequence SEQ ID No. 5 or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 5;
  - the CDR of sequence SEQ ID No. 2, 7, 9 or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 2, 7, 9;
  - 25       - the CDR of sequence SEQ ID No. 4, 11 or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 4, 11; and
  - the CDR of sequence SEQ ID No. 6, 12 or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 6, 12.
  - 30

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  
5 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.

10 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.

15 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  
20 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.

25 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')<sub>2</sub>, 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  
30 example, of the fragments Fv, scFv (sc=simple chain), Fab, F(ab')<sub>2</sub>, 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')<sub>2</sub>-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  
5 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  
10 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')<sub>2</sub>,  
15 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  
20 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.

The invention also targets the original murine antibody, namely an antibody according to the invention, or its derived compounds or functional fragments,  
25 characterized in that said antibody is a murine antibody and in that it comprises a light-chain of amino acid sequence SEQ ID No. 15, or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 15, and a heavy-chain of amino acid sequence SEQ ID No. 16, or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal  
30 alignment with sequence SEQ ID No. 16.

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

Table 2 (wherein Mu. = murine and Hu. = humanized)

| Antibody | CDR numbering | Heavy chain              | Light chain         | SEQ ID NO. |
|----------|---------------|--------------------------|---------------------|------------|
| 6F4      | Common        |                          | CDR-L1              | 1          |
|          |               |                          | CDR-L2              | 3          |
|          |               |                          | CDR-L3              | 5          |
|          |               | CDR-H1                   |                     | 2          |
|          |               | CDR-H2                   |                     | 4          |
|          |               | CDR-H3                   |                     | 6          |
|          | IMGT          |                          | CDR-L1              | 1          |
|          |               |                          | CDR-L2              | 3          |
|          |               |                          | CDR-L3              | 5          |
|          |               | CDR-H1                   |                     | 7          |
|          |               | CDR-H2                   |                     | 4          |
|          |               | CDR-H3                   |                     | 12         |
|          | Kabat         |                          | CDR-L1              | 8          |
|          |               |                          | CDR-L2              | 10         |
|          |               |                          | CDR-L3              | 5          |
|          |               | CDR-H1                   |                     | 9          |
|          |               | CDR-H2                   |                     | 11         |
|          |               | CDR-H3                   |                     | 6          |
|          |               |                          | Mu. variable domain | 13         |
|          |               | Mu. variable domain      |                     | 14         |
|          |               |                          | Mu. entire          | 15         |
|          |               | Mu. entire               |                     | 16         |
|          |               |                          | Hu. variable domain | 17         |
|          |               | Hu. variable domain (V1) |                     | 18         |
|          |               | Hu. variable domain (V2) |                     | 19         |

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

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

According to another aspect, the invention relates to a murine hybridoma capable of secreting a monoclonal antibody according to the invention, notably the

hybridoma of murine origin filed with the French center for microorganism cultures (CNCM, Pasteur Institute, Paris, France) on July 6, 2006, under number I-3646. Said hybridoma was obtained by the fusion of Balb/C immunized mice splenocytes and cells of the myeloma Sp 2/O-Ag 14 lines.

5           The monoclonal antibody, here referred to as 6F4, or its derived compounds or functional fragments, characterized in that said antibody is secreted by the hybridoma filed with the CNCM on July 4, 2006, under number I-3646, obviously forms part of the present invention.

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

10           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.

          “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; Verhoeyn *et al.*, Science, 239:1534-1536, 1988; Riechmann *et al.*, Nature, 332:323-327, 1988).

30           The humanized antibodies of the invention or fragments of same can be prepared by techniques known to a person skilled in the art (such as, for example, those described in the documents Singer *et al.*, J. Immun., 150:2844-2857, 1992; Mountain *et al.*,

Biotechnol. Genet. Eng. Rev., 10:1-142, 1992; and Bebbington *et al.*, Bio/Technology, 10:169-175, 1992). Such humanized antibodies are preferred for their use in methods involving *in vitro* diagnoses or preventive and/or therapeutic treatment *in vivo*. Other humanization techniques, also known to a person skilled in the art, such as, for example, the "CDR grafting" technique described by PDL in patents EP 0 451 261, EP 0 682 040, EP 0 939 127, EP 0 566 647 or US 5,530,101, US 6,180,370, US 5,585,089 and US 5,693,761. US patents 5,639,641 or 6,054,297, 5,886,152 and 5,877,293 can also be cited.

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

More particularly, the humanization method for the 6F4 antibody is described in detail in examples 2 and 3 for the light and heavy chains, respectively.

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

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

In another aspect of the invention, the applicant has also identified the antigen recognized by the antibody according to the invention.

The method used to accomplish this is described in detail in example 4 below.

JAM-A is a membrane protein belonging to the immunoglobulin superfamily (IgSF), in which it belongs to the junctional adhesion molecule (JAM) family. In man, the JAM family comprises several members, including the JAM-A, JAM-B, JAM-C, A33 and A34 proteins. Among the members of the JAM family, JAM-A has the highest homology with JAM-B and JAM-C, approximately 35% sequence identity in amino acids and 45% similarity with these two proteins. JAM-A protein is also called JAM A, F11R, F11 receptor, JAM-1, JAM 1, PAM-1 or CD321.

Two isoforms of the JAM-A precursor differing by the length of the extracellular region were identified:

- isoform a: 299 amino acids (SEQ ID No. 61)

- isoform b: 259 amino acids (SEQ ID No. 63).

The nucleotide sequences of the two isoforms are represented with SEQ ID No. 62 for isoform a and SEQ ID No. 64 for isoform b.

The protein expressed on the surface of the human cells has a single polypeptide chain with an intracellular C-terminal domain, a single transmembrane domain (21 amino acids) and an N-terminal extracellular region containing two "Ig-like" domains.

JAM-A has an N-glycosylation site, an Asn residue in position 185 for isoform a and 145 for isoform b, and two disulfide bridges, one between Cys residues 50 and 109 in the Ig N-terminal domain and one between residues Cys 153 and 212 in the second Ig domain.

The presence of the two extracellular Ig-like domains was confirmed by crystallography (Kostrewa *et al.*, 2001, EMBO J. 16:4391-4398; Prota *et al.*, 2003, Proc. Natl. Acad. Sci. USA, 100:5366-5371). These two domains are connected by a tripeptide linker (sequence VLV [127-129], isoform A). These structural studies also confirmed the implication of JAM-A in homophilic interactions on the cell surface involving the extracellular region; this region, produced in recombinant form and capable of forming homodimers in solution (Bazzoni *et al.*, 2000, J. Biol. Chem. 275:30970-30976) also made it possible to identify the amino acids involved in these interactions: Arg 59, Glu 61, Lys 63, Leu 72, Tyr 75, Met 110, Glu 114, Tyr 119 and Glu 121. The tripeptide RVE [59-61] is relatively conserved within the JAM family (RLE for JAM-B, RIE for JAM-C) and constitutes the minimal motif for homodimer formation (Kostrewa *et al.*, 2001, EMBO J. 16:4391-4398).

In epithelial and endothelial cells, JAM-A is mainly found in the tight junctions (Liu *et al.*, 2000, J. Cell Sci., 113:2363-2374). The cytoplasmic region contains a type II PDZ domain in the C-terminal position (sequence FLV [298-300], isoform a, which is responsible for the interaction of JAM-A with various cytosolic proteins associated with the tight junction, also containing a PDZ domain, such as ZO-1, AF-6, MUPP-1 and PAR-3 (Ebnet *et al.*, 2000, J. Biol. Chem., 275:27979-27988; Itoh *et al.*, 2001, J. Cell Biol., 154:491-498; Hamazaki *et al.*, 2002, J. Biol. Chem., 277:455-461). Murine antibodies directed against the region [111-123] involved in dimer formation, so-called J3F.1 and J10.4 antibodies, are capable of inhibiting the homodimerization of JAM-A

and the reconstruction of the epithelial barrier *in vitro* (Mandell *et al.*, 2004, J. Biol. Chem., 279:16254-16262).

JAM-A interacts with integrin  $\alpha_v\beta_3$  and is involved in the migration of endothelial cells on vitronectin, ligand of integrin  $\alpha_v\beta_3$  (Naik and Naik, 2005, J. Cell Sci. 119:490-499). AntiJAM-A antibody J3F.1, in the same manner as an anti- $\alpha_v\beta_3$  antibody, inhibits the migration of endothelial cells and the angiogenesis induced by bFGF *in vitro* (Naik *et al.*, 2003, Blood, 102:2108-2114). Various signaling pathways were demonstrated in endothelial cells: MAP kinases, PI3-kinase and PKC (Naik et Naik, 2005, J. Cell Sci., 119:490-499; Naik *et al.*, 2003, Blood, 102:2108-2114; Naik *et al.*, 2003, Arterioscler. Thromb. Vasc. Biol., 23:2165-2171).

JAM-A is also expressed in monocytes, lymphocytes, neutrophils and platelets (Williams *et al.*, 1999, Mol. Immunol., 36:1175-1188). JAM-A protein was however initially identified as a receptor of the F11 antibody, an antibody capable of activating platelets and inducing their aggregation (Naik *et al.*, 1995, Biochem. J., 310:155-162; Sobocka *et al.*, 2000, Blood, 95:2600-2609). Peptides [28-60] and [97-109] belong to the F11 antibody epitope and are involved in platelet activation and aggregation phenomena and in homodimerization (Babinska *et al.*, 2002, Thromb. Haemost., 87:712-721).

Rat antibody BV11, directed against the murine form of JAM-A, inhibits the trans-endothelial migration of monocytes *in vitro* and *in vivo* (Del Maschio *et al.*, 1999, J. Exp. Med., 190:1351-1356). Ostermann and colleagues (2002, Nature Immunol., 3:151-158) showed that JAM-A was a ligand of  $\alpha_L\beta_2$  or LFA-1 (lymphocyte function-associated antigen 1) integrin, which is overexpressed in response to certain chemokines during the development of an anti-inflammatory response and is required for the diapedesis or migration of leukocytes to the site of inflammation. JAM-A, via the second Ig-like domain, contributes to the adhesion and trans-endothelial migration of T lymphocytes and neutrophils (Ostermann *et al.*, 2002, Nature Immunol., 3:151-158), and thus plays an important role in the recruitment of leukocytes to the site of inflammation.

JAM-A protein is also implicated in viral infection phenomena. JAM-A is indeed a receptor of reovirus, viruses responsible for certain types of encephalitis by means of interacting with attachment protein  $\sigma 1$  (Barton *et al.*, 2001, Cell 104:441-451).

AntiJAM-A antibody J10.4 inhibits the binding of reovirus to JAM-A (Forrest *et al.*, 2003, J. Biol. Chem., 278:48434-48444).

To date, none of the antibodies mentioned above directed against the human form of JAM-A exhibit activity *in vivo*, much less antitumor activity. Such antibodies  
5 are used only as research tools. Thus, in the former art, there is a genuine lack of an antitumor antibody active *in vitro* and *in vivo*.

According to a specific aspect, the antibody of the invention, or its derived compounds or functional fragments, is characterized in that it is capable of specifically binding to JAM-A protein (according to the English nomenclature "Junctional Adhesion  
10 Molecules").

According to still another aspect, the antibody of the invention, or its derived compounds or functional fragments, is characterized in that it exhibits a  $K_D$  for JAM-A between roughly 1 nM and roughly 1 pM. More preferably, said  $K_D$  for JAM-A is between roughly 10 pM and roughly 40 pM.

15 The expression " $K_D$ " refers to the dissociation constant of a given antibody-antigen complex.  $K_D = K_{off}/K_{on}$  with  $K_{off}$  consisting of the "off rate" constant for the dissociation of the antibody from the antibody-antigen complex and  $K_{on}$  consisting of the level at which the antibody binds the antigen (Chen Y. *et al.*, 1999, J.Mol.Biol., 293:865-881).

20 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 according to the invention, or one of its derived compounds or functional fragments;

25 b) a nucleic acid complementary to a nucleic acid as defined in a);

c) a nucleic acid of at least 18 nucleotides capable of hybridizing under highly stringent conditions with at least one of the CDRs of nucleic acid sequences SEQ ID Nos. 20 to 31 or with a sequence with at least 80%, preferably 85%, 90%, 95% and 98%, identity after optimal alignment with sequence SEQ ID Nos. 20 to 31; and

30 d) a nucleic acid of at least 18 nucleotides capable of hybridizing under highly stringent conditions with at least the light chain of nucleic acid sequence SEQ ID No. 32 or 36 and/or the heavy chain of nucleic acid sequence SEQ ID No. 33, 37 or 38,

or with a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 32 or 36 and/or 33, 37 or 38.

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

5

Table 3

| Antibody | CDR numbering | Heavy chain              | Light chain         | SEQ ID NO. |
|----------|---------------|--------------------------|---------------------|------------|
| 6F4      | Common        |                          | CDR-L1              | 20         |
|          |               |                          | CDR-L2              | 22         |
|          |               |                          | CDR-L3              | 24         |
|          |               | CDR-H1                   |                     | 21         |
|          |               | CDR-H2                   |                     | 23         |
|          |               | CDR-H3                   |                     | 25         |
|          | IMGT          |                          | CDR-L1              | 20         |
|          |               |                          | CDR-L2              | 22         |
|          |               |                          | CDR-L3              | 24         |
|          |               | CDR-H1                   |                     | 26         |
|          |               | CDR-H2                   |                     | 23         |
|          |               | CDR-H3                   |                     | 27         |
|          | Kabat         |                          | CDR-L1              | 28         |
|          |               |                          | CDR-L2              | 29         |
|          |               |                          | CDR-L3              | 24         |
|          |               | CDR-H1                   |                     | 30         |
|          |               | CDR-H2                   |                     | 31         |
|          |               | CDR-H3                   |                     | 25         |
|          |               |                          | Mu. variable domain | 32         |
|          |               | Mu. variable domain      |                     | 33         |
|          |               |                          | Mu. entire          | 34         |
|          |               | Mu. entire               |                     | 35         |
|          |               |                          | Hu. variable domain | 36         |
|          |               | Hu. variable domain (V1) |                     | 37         |
|          |               | Hu. variable domain (V2) |                     | 38         |

The terms “nucleic acid”, “nucleic sequence”, “nucleic acid sequence”,  
 10 “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  
5 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  
10 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  
15 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  
20 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)  
25 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%  
30 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).

5           The invention also relates to a vector comprising anucleic 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  
10       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  
15       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  
20       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  
25       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  
30       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:

5 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  
10 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 an vector according to the invention is cultured under conditions that allow the expression of the aforesaid polypeptide and recovery of said recombinant  
15 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. An vector according to the invention carrying such a sequence can thus be used  
20 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  
25 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  
30 the invention.

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

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

According to a novel embodiment, the invention relates to an antibody, or its derived compounds or functional fragments, consisting of an antibody that is bispecific  
10 in the sense that it comprises a second motif capable of interacting with any receptor implicated in the development of tumors, such as, for example, VEGFR, VEGF, EGFR, IGF-1R, HER2neu, HGF, cMET, FGF, tetraspanins, integrins, CXCR4 or CXCR2.

According to a first embodiment, one such antibody consists of a bispecific antibody and comprises a second motif that specifically inhibits the binding of EGF  
15 with human epidermal growth factor receptor (EGFR) and/or specifically inhibiting the tyrosine kinase activity of said EGFR. According to an even more preferred aspect of the invention, said second antiEGFR motif arises from the monoclonal antibody cetuximab (C225 or erbitux), matuzumab, huR3, HuMax-EGFR or panitumab.

According to a second embodiment, the antibody according to the invention  
20 consists of a bispecific antibody and comprises a second motif specifically inhibiting the activity modulated by the HER2/neu receptor and/or specifically inhibiting the tyrosine kinase activity of said HER2/neu receptor. More particularly, said second antiHER2/neu motif arises from the mouse monoclonal antibody 4D5 or 2C4 or from the humanized antibody trastuzumab or pertuzumab.

25 According to a third embodiment, the antibody according to the invention consists of a bispecific antibody and comprises a second motif specifically inhibiting the binding of hepatocyte growth factor (HGF) with the cMET receptor and/or specifically inhibiting the tyrosine kinase activity of said cMET receptor.

According to a fourth embodiment, the antibody according to the invention  
30 consists of a bispecific antibody and comprises a second motif specifically inhibiting the activity modulated by the IGF-1R receptor and/or specifically inhibiting the tyrosine kinase activity of said IGF-1R receptor. More particularly, said second antiIGF-1R

motif arises from mouse monoclonal antibody 7C10, from corresponding humanized antibody h7C10 (Goetsch *et al.*, international patent application WO 03/059951), from hEM164 antibodies (Maloney *et al.*, Cancer Res., 2003, 63 (16):5073-5083), from the antiIGF-1R antibodies developed by Abgenix (see US patent application 2005/281812)  
5 or from Mab 39, 1H7 (Li *et al.*, Cancer Immunol. Immunother., 2000, 49(4-5):243-252) or 4G11 (Jackson-Booth *et al.*, Horm. Metab. Res., 2003, 35(11-12):850-856).

Lastly, according to a final embodiment, the antibody of the invention consists in a bispecific antibody and comprises a second motif capable of interacting with any type of receptor implicated in tumor development, such as, as non-limiting examples,  
10 VEGFR, VEGF, FGF (fibroblast growth factor) or any member of the CXCR (chemokine receptor) family, such as CXCR2 or CXCR4.

Also suitable for mention are antiCD20 antibodies such as a rituximab, ibritumomab or tositumomab; antiCD33 antibodies such as gemtuzumab or lintuzumab; antiCD22 antibodies such as epratuzumab; antiCD52 antibodies such as alemtuzumab;  
15 antiEpCAM antibodies such as edrecolomab, Ch 17-1A or IGN-101; antiCTP21 or 16 antibodies such as Xactin; antiDNA-Ag antibodies such as <sup>131</sup>I-Cotara TNT-1; antiMUC1 antibodies such as pemtumomab or R1150; antiMUC18 antibodies such as ABX-MA1; antiGD3 antibodies such as mitumomab; antiECA antibodies such as CeaVac or labetuzumab; antiCA125 antibodies such as OvaRex; antiHLA-DR  
20 antibodies such as apolizumab; antiCTLA4 antibodies such as MDX-010; antiPSMA antibodies such as MDX-070, <sup>111</sup>In & <sup>90</sup>Y-J591, <sup>177</sup>Lu J591, J591-DM1; antiLewis Y antibodies such as IGN311; antiangiogenesis antibodies such as AS1405 and 90YmuBC1; antiTrail-R1 antibodies such as TRAIL R1mAb or TRAIL R2mAb.

The bispecific or bifunctional antibodies constitute a second generation of  
25 monoclonal antibodies in which two different variable regions are combined in the same molecule (Hollinger and Bohlen, 1999, Cancer and metastasis, rev. 18:411-419). Their utility was demonstrated in both diagnostic and therapeutic domains relative to their capacity to recruit new effector functions or to target several molecules on the surface of tumor cells; such antibodies can be obtained by chemical methods (Glennie MJ *et al.*,  
30 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,  
5 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  
10 antibodies, the use of such bispecific antibodies has the advantage of reducing the treatment's toxicity. Indeed, the use of a bispecific antibody makes it possible to decrease the overall quantity of circulating antibodies and, consequently, possible toxicity.

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

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

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

According to still another embodiment, the present invention also relates to a pharmaceutical composition as described above that comprises at least a second antitumor compound selected among the compounds capable of specifically inhibiting  
25 the tyrosine kinase activity of receptors such as IGF-IR, EGFR, HER2/neu, cMET, VEGFR or VEGF, or any other antitumor compound known to a person skilled in the art. In a second preferred aspect of the invention, said second compound can be selected among the antibodies antiEGFR, antiIGF-IR, antiHER2/neu, anticMET, VEGFR, VEGF, etc., isolated, or their functional fragments and derived compounds, capable of  
30 inhibiting the proliferative and/or anti-apoptotic and/or angiogenic and/or inductive activity of metastatic dissemination promoted by said receptors.

According to still another embodiment of the invention, the composition comprises, in addition, as a combination product for use in a simultaneous, separated or extended fashion, at least one inhibitor of the tyrosine kinase activity of receptors such as IGF-IR, EGFR, HER2/neu, cMET and VEGFR.

5 In another preferred embodiment, said inhibitor of the tyrosine kinase activity of these receptors is selected from the group comprising derived natural agents, dianilinophthalimides, pyrazolo- or pyrrolo-pyridopyrimidines or quinazolines. Such inhibiting agents, well-known to a person skilled in the art, are described in the literature (Ciardiello F., Drugs 2000, Suppl. 1, 25-32).

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

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

15 "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.

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

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

Such agents, for example, are cited in VIDAL, on the page devoted to compounds related to oncology and hematology under the heading "Cytotoxic"; the cytotoxic compounds cited by reference to this document are cited herein as preferred cytotoxic agents.

5 "Alkylating agent" refers to any substance that can bind covalently with or can alkylate any molecule, preferentially a nucleic acid (e.g., DNA), within a cell. Examples of such alkylating agents include nitrogen mustards such as mechlorethamine, chlorambucil, melphalan, chlorhydrate, pipobroman, prednimustine, disodium phosphate or estramustine; oxazaphosphorines such as cyclophosphamide, altretamine, 10 trofosfamide, sulfofosfamide or ifosfamide; aziridines or ethylene-imines such as thiotepa, triethylenamine or altetramine; nitrosoureas such as carmustine, streptozocine, fotemustine or lomustine; alkyl sulfonates such as busulfan, treosulfan or improsulfan; triazenes such as dacarbazine; or platinum complexes such as cisplatin, oxaliplatin or carboplatin.

15 "Antimetabolite" refers to a substance that blocks growth and/or cellular metabolism by interfering with certain activities, generally DNA synthesis. Examples of antimetabolites include methotrexate, 5-fluorouracil, floxuridine, 5-fluorodeoxyuridine, capecitabine, cytarabine, fludarabine, cytosine arabinoside, 6-mercaptopurine (6-MP), 6-thioguanine (6-TG), chlorodesoxyadenosine, 5-azacytidine, 20 gemcitabine, cladribine, deoxycoformycin and pentostatin.

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

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

"Chromatin inhibitors" or "topoisomerase inhibitors" are substances that inhibit 30 the normal functioning of proteins that shape chromatin, such as topoisomerases I and II. Examples of such inhibitors include, for topoisomerase I, camptothecin and its

derivatives, such as irinotecan or topotecan; for topoisomerase II, etoposide, etiposide phosphate and teniposide.

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

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

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

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

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

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

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

In order to facilitate binding between said cytotoxic agent and the antibody according to the invention, spacer molecules can be introduced between the two

compounds to bind, such as the poly(alkylene)glycol polyethyleneglycol or the amino acids; or, in another embodiment, said cytotoxic agents' active derivatives, into which have been introduced functions capable of reacting with said antibody, can be used. These binding techniques are well-known to a person skilled in the art and will not be discussed in more detail in the present description.

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

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

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

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

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

"Toxin or radioisotope combined with at least one antibody of the invention, or a functional fragment of same" refers to any means that makes it possible to bind said

toxin or said radioisotope to that at least one antibody, notably by covalent binding between the two compounds, with or without the introduction of the binding molecule.

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

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

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

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

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

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

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

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

10 In a preferred manner, said cancer is a cancer chosen among estrogen-related breast cancer, non-small cell lung cancer, colon cancer and/or pancreatic cancer.

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

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

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

b) demonstrating the antigen-antibody complex possibly formed.

Thus, the present invention also comprises the kits or accessories for the  
25 implementation of a method as described (for detecting the expression of a gene from *Legionella pneumophila* Paris or from an associated organism, or for detecting and/or identifying *Legionella pneumophila* Paris bacteria or associated microorganisms), comprising the following elements:

a) a polyclonal or monoclonal antibody of the invention;

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

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

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

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

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

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

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

Others conjugates can also include chemiluminescent labels such as luminol and dioxetane, bioluminescent labels such as luciferase and luciferin, or radioactive labels

such as iodine<sup>123</sup>, iodine<sup>125</sup>, iodine<sup>126</sup>, iodine<sup>133</sup>, bromine<sup>77</sup>, technetium<sup>99m</sup>, indium<sup>111</sup>, indium<sup>113m</sup>, gallium<sup>67</sup>, gallium<sup>68</sup>, ruthenium<sup>95</sup>, ruthenium<sup>97</sup>, ruthenium<sup>103</sup>, ruthenium<sup>105</sup>, mercury<sup>107</sup>, mercury<sup>203</sup>, rhenium<sup>99m</sup>, rhenium<sup>101</sup>, rhenium<sup>105</sup>, scandium<sup>47</sup>, tellurium<sup>121m</sup>, tellurium<sup>122m</sup>, tellurium<sup>125m</sup>, thulium<sup>165</sup>, thulium<sup>167</sup>, thulium<sup>168</sup>, fluorine<sup>18</sup>, yttrium<sup>199</sup> and iodine<sup>131</sup>. Existing methods known to a person skilled in the art for binding radioisotopes with antibodies, either directly or via a chelating agent such as the EDTA or DTPA mentioned above, can be used for as diagnostic radioisotopes. Thus should be mentioned labeling with [<sup>125</sup>I]Na by the chloramine-T technique [Hunter W.M. and Greenwood F.C. (1962) *Nature* 194:495]; labeling with technetium<sup>99m</sup> as described by Crockford *et al.* (US patent 4,424,200) or bound via DTPA as described by Hnatowich (US patent 4,479,930).

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

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

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

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

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

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

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

5 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.

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

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

15

#### FIGURE LEGENDS

Figure 1 shows the respective sequences of the heavy and light chains of the murine 6F4 antibody. CDRs are underlined and in bold (according to the Kabat numbering).

20 Figures 2A and 2B represent the respective alignments of the V (figure 2A) and J (figure 2B) regions of murine 6F4 antibody and the murine cell lines selected, namely IGKV19-93\*01 (SEQ ID No. 39) for the V region and IGKJ1\*01 (SEQ ID No. 40) for the J region.

Figures 3A and 3B represent the respective alignments of the V (figure 3A) and  
25 J (figure 3B) regions of murine 6F4 antibody and the human cell lines selected, namely IGKV1-33\*01 (SEQ ID No. 41) for the V region and IGKJ1\*01 (SEQ ID No. 42) for the J region.

Figure 4 represents the protein sequence of the light chain of the 6F4 antibody with reference to the respective KABAT and IMGT numbering systems.

30 Figures 5A, 5B and 5C represent the respective alignments of the V (figure 5A), D (figure 5B) and J (figure 5C) regions of the murine 6F4 antibody and the murine cell lines selected, namely IGHV1S135\*01 (SEQ ID No. 43) for the V region, IgHD-

ST4\*01 (SEQ ID No. 44) for the D region and IgHJ2\*01 (SEQ ID No. 45) for the J region.

Figures 6A, 6B and 6C represent the respective alignments of the V (figure 6A), D (figure 6B) and J (figure 6C) regions of the murine 6F4 antibody and the human cell lines selected, namely IGHV1-f\*01 (SEQ ID No. 46) for the V region, IGHD1-1\*01 (SEQ ID No. 47) for the D region and IGHJ4\*01 (SEQ ID No. 48) for the J region.

Figure 7 represents the protein sequence of the heavy chain of the 6F4 antibody with reference to the respective KABAT and IMGT numbering systems.

Figures 8A and 8B represent the 6F4-sepharose immunopurification of 6F4 antigen from HT-29 cell membranes. Analyses of fractions collected by SDS-PAGE electrophoresis (figure 8A) and western blot (figure 8B) are presented as well.

Figures 9A and 9B present an analysis by SDS-PAGE electrophoresis (figure 9A) and western blot (figure 9B) of immunopurified protein. Two purifications (#1 and #2) were performed and analyzed under reducing and in non-reducing conditions.

Figure 10 presents an analysis by MALDI-TOF mass spectrometry of the mixture of peptides extracted after tryptic hydrolysis.

Figures 11A and 11B consist of the confirmation of a protein identified by western blot (non-reducing conditions): revealed using 6F4 antibody (figure 11A) and anti-human JAM-A polyclonal antibody (figure 11B).

Figure 12 shows the specificity of the 6F4 antibody for human JAM-A protein. The quantities deposited for each protein are 250 ng, 25 ng and 10 ng.

Figure 13 represents sensorgrams obtained after 2 minutes of injection (double arrow) of the 6F4 antibody at 100 nM in HBS-EP buffer on murine JAM1 Fc protein (Flow cell #1, bottom graph) and on murine JAM1 Fc protein (Flow cell #2, top graph) with a dissociation time at 25°C of 5 minutes and a flow rate of 30 µl/min (CM4: m-JAM1-Fc 501.6 RU (Fc1) and 511.5 RU (Fc2)).

Figure 14 represents sensorgrams obtained with a double reference, (Fc2-Fc1)6F4(Fc2-Fc1)HBS-EP. The curve is fitted using a Langmuir A+B binding model. The calculated kinetic parameters (black curve) are as follows:  $k_a = (1.38 \pm 0.001) \cdot 10^5 \text{ M}^{-1} \text{ s}^{-1}$ ;  $k_d = (0.25 \pm 1.58) \cdot 10^{-6} \text{ s}^{-1}$ ;  $R_{\text{max}} (\text{global fitting}) = 371 \text{ RU}$ ;  $\kappa^2 = 0.853$ .

Figure 15 illustrates the antitumor activity of the 6F4 antibody in a xenograft model of MCF-7 cells in the Swiss nude mouse. The 6F4 antibody was tested by IP

route in unpurified form (peritoneal cavity fluid), at the theoretical dose of 250 µg/mouse, twice per week. The 9G4 antibody is an antibody of the same isotype (IgG1), non-relevant with respect to the activity measured.

Figure 16 illustrates JAM-A protein expression recognized by Mab 6F4 on the surface of various tumor lines.

Figure 17 represents the sequence of the humanized 6F4 VL domain wherein: \* correspond to amino acids changed *de facto* to their human counterparts, 1 correspond to amino acids analysed for their abilities to be humanized, the human residue being indicated below the sign, and 2 correspond to amino acids that remain murin in the humanized 6F4 VL domain.

Figure 18 represents the sequence of the humanized 6F4 VH domain wherein: \* correspond to amino acids changed *de facto* to their human counterparts, 1 correspond to amino acids analysed for their abilities to be humanized, the human residue being indicated below the sign, and 2 correspond to amino acids that remain murin in the humanized 6F4 VH domain.

Figure 19 illustrates the *in vitro* JAM-A down-regulation induced by the 6F4 MAb.

Figure 20 illustrates the *in vivo* inhibition of tumor cell proliferation induced by the 6F4 MAb.

Figure 21 represents the *in vivo* down-regulation of JAM-A by the 6F4 Mab.

Figure 22 represents curves of the Comparison of 6F4 and its F(ab')<sub>2</sub> fragment on the MCF-7 *in vivo* model.

Figure 23 illustrates the comparison of normal versus tumoral expression of JAM-A on thyroid tissues.

Figure 24 illustrates the comparison of normal versus tumoral expression of JAM-A on lung tissues.

Figure 25 illustrates the comparison of normal versus tumoral expression of JAM-A on Breast tissues.

Figure 26 represents curves illustrating the *in vivo* activity of 6F4 on A431 epidermoid carcinoma xenograft in nude mice.

Figure 27 illustrates the effect of the 6F4 antibody on A. non specific lymphoproliferation induced with PHA and B. antigen presentation process. First experiment with 2 independent donors.

Figure 28 illustrates the effect of the 6F4 antibody on A. non specific lymphoproliferation induced with PHA and B. antigen presentation process. Second experiment with 2 independent donors.

Figure 29 illustrates the platelet aggregation on 10 human normal donors. Results are expected as mean $\pm$  sd.

Figure 30 represents the serotonin release on 10 human normal donors. Results are expected as mean $\pm$  sd.

Figure 31 represents the alignment of the 6F4 VH domain and IGHV1-03\*01 germline gene (SEQ ID No. 49).

## EXAMPLES

### Example 1: Generation of the 6F4 antibody

To generate the murine monoclonal antibody (Mab), BALB/C mice were immunized using  $5 \times 10^6$  MCF-7 cells from ATCC. After a final booster injection of  $10^7$  MCF-7 cells, cells from lymph nodes of mice are fused with Sp2/O-Ag14 myeloma cells using the techniques classically described by Kohler and Milstein. The supernatants of the hybridomas arising from the fusion were then screened for functional activity, namely the inhibition of the proliferation of MCF-7 cells *in vitro*.

For this screening, MCF-7 cells are cultured in 96-well culture dishes at  $5 \times 10^3$  cells/well in 100  $\mu$ l of hybridoma medium without fetal calf serum. The plates are incubated for 24 hours at 37°C under an atmosphere of 5% CO<sub>2</sub>. After 24 hours, 50  $\mu$ l of the supernatant of the hybridomas to be screened are added to each well. The last line on the plate is reserved for the controls:

- three wells are supplemented by 50  $\mu$ l of a hybridoma supernatant that is non-relevant with respect to the activity sought and that is cultured in the same culture medium as that used for the fused cells. These wells will be used to calibrate the impact of inactive supernatant on the incorporation of tritiated thymidine;
- three wells will receive 50  $\mu$ l of hybridoma culture medium.

After roughly 52 hours of culture, each well is supplemented by 0.25  $\mu$ Ci of [ $^3$ H]thymidine and incubated again for 20 hours at 37°C. The incorporation of [ $^3$ H]thymidine in the DNA, indicating cell proliferation, is quantified by measuring liquid scintillation. Background noise and thresholds are determined for each plate as a function of the control wells containing the medium alone and the non-relevant hybridoma supernatant.

By this method, 43 hybridomas secreting antibodies inhibiting the growth of MCF-7 cells were selected after a first screening. Eleven of these 43 hybridomas had weak or non-existent growth and were abandoned. During proliferation tests performed following the expansion and cloning of the hybridomas, only the hybridomas whose supernatant had a  $\geq 20\%$  inhibiting activity on the proliferation of MCF-7 cells were selected. At the end of the cloning/selection process, only one clone proved to have the required properties, the 6F4 clone.

Example 2: Process of humanization by CDR-grafting of the variable region of the light chain of the 6F4 antibody (6F4 VL)

*a) Comparison of the 6F4 VL nucleotide sequence with all known murine cell line sequences*

As a preliminary step in humanization by CDR-grafting, the 6F4 VL nucleotide sequence initially was compared with all of the murine cell line sequences present in the IMGT data bank (Internet address: <http://imgt.cines.fr>).

Regions V and J of mouse cell lines having a sequence identity of 98.56% for the V region and 100% for the J region were identified, respectively IGKV19-93\*01 (SEQ ID No. 39, EMBL nomenclature: AJ235935) and IGKJ1\*01 (SEQ ID No. 40, EMBL nomenclature: V00777).

Considering these identity percentages, it was decided to use the 6F4 VL sequence directly.

These alignments are represented in figure 2A for the V region and in figure 2B for the J region.

*b) Comparison of the nucleotide sequence of 6F4 VL with all known human cell line sequences*

In order to identify the best human candidate for CDR-grafting, the human-origin germline having the greatest possible identity with 6F4 VL was sought. For this purpose, the nucleotide sequence of mouse 6F4 VL was compared with all of the human cell line sequences present in the IMGT data base.

- 5       Regions V and J of human-origin cell lines were identified with a sequence identity of 81.36% for the V region, namely IGKV1-33\*01 (SEQ ID No. 41, EMBL nomenclature: M64856) and 86.84% for the J region, namely IGKJ1\*01 (SEQ ID No. 42, EMBL nomenclature: J00242).

- 10       Cell lines IGKV1-33\*01 for the V region and IGKJ1\*01 for the J region were thus selected as human receptor sequences for mouse 6F4 VL CDRs.

These alignments are presented in figure 3A for the V region and in figure 3B for the J region.

*c) Humanized versions of 6F4 VL*

- 15       The following step in the humanization process consists of joining together the IGKV1-33\*01 and IGKJ1\*01 cell line sequences and then joining the mouse 6F4 VL CDRs to the scaffold regions of these same germlines.

- 20       This stage of the process the molecular model of the mouse 6F4 Fv regions will be particularly useful in the choice of the mouse residues to preserve because they may play a role either in maintaining the molecule's three-dimensional structure (canonical structure of CDRs, VH/VL interfaces, etc.) or in binding the antigen. In the scaffold regions, each difference between mouse (6F4 VL) and human (IGKV1-33\*01/IGKJ1\*01) nucleotides will be examined very carefully.

For more clarity in the following, figure 4 presents the 6F4VL sequence with reference to KABAT and IMGT classifications.

- 25       Three murine residues were identified which must be preserved.

Residue 33 (Ile) takes part in CDR1 anchoring according to IMGT and is part of CDR1 according to Kabat.

Residue 49 (His) takes part in CDR2 anchoring according to IMGT, takes part in the VH/VL interface and belongs to the Vernier zone.

- 30       Residue 53 (Thr) takes part in CDR2 anchoring according to IMGT and is part of CDR2 according to Kabat.

Initially, three changes in the scaffold regions of IGKV1-33\*01 and IGKJ1\*01 will be studied. These changes relate to residues 24, 69 and 71 (IMGT nomenclature). It should be understood, of course, that these three changes will be studied independently of each other and also in various combinations. The aim is to have available all possible mutants in order to test them and to select the mutant that has preserved the best binding properties. ELISA/Biacore binding tests will thus be performed on each mutant.

Residue 24 (Lys/Gln) is near CDR1 and could as a result be critical for maintaining a conformation that enables proper CDR1 presentation. More particularly, this residue is likely to interact with residues 69-70 within the Vernier zone. Lys is only slightly represented in human VLs but is part of CDR1 according to Kabat.

Although residue 69 (Arg/Thr) is in the Vernier zone and thus directly takes part in CDR1's canonical structure, this residue is always Thr in the human VL.

Although residue 71 (Tyr/Phe) directly takes part in CDR1's canonical structure, it is systematically Phe in the human VL.

Secondly, a modification of residue 56 (Ala) into Thr can be considered. This residue, although outside of CDRs according to IMGT, belongs to CDR2 according to Kabat.

Third and last, two additional changes could be made at residues 34 and 55 (IMGT nomenclature). The two residues, outside of the CDRs defined IMGT, are included in the CDRs defined by Kabat.

Residue 34 (Ala/Asn) belongs to CDR1 according to Kabat and takes part in the VH/VL interface. Such a mutation remains relevant in spite of the strong representation of Ala in man.

Residue 55 (Gln/Glu) is part of CDR2 according to Kabat and also takes part in the VH/VL interface. Such a mutation also remains relevant in spite of the strong representation of Gln in man.

As was described above, these three mutations could be tested independently or in various combinations.

Example 3: Process of humanization by CDR-grafting of the variable region of the heavy chain of the 6F4 antibody (6F4 VH)

a) *Comparison of the 6F4 VH nucleotide sequence with all known murine cell line sequences*

As a preliminary step in humanization by CDR-grafting, the 6F4 VH nucleotide sequence initially was compared with all of the murine cell line sequences present in the  
5 IMGT data bank (Internet address: <http://imgt.cines.fr>).

Regions V, D and J of murine cell lines having a sequence identity of 99.30% for the V region (IGHV1S135\*01; SEQ ID No. 43; EMBL nomenclature: AF304556), of 80% for the D region (IGHD-ST4\*01; SEQ ID No. 44; EMBL nomenclature: M23243) and of 100% for the J region (IGHJ2\*01; SEQ ID No. 45; EMBL  
10 nomenclature: V00770).

These alignments are represented in figure 5A for the V region, figure 5B for the D region and figure 5C for the J region.

Considering these identity percentages, it was decided to use the 6F4 VH sequence directly, as was the case for 6F4 VL.

15 b) *Comparison of the nucleotide sequence of 6F4 VH with all known human cell line sequences*

In order to identify the best human candidate for CDR-grafting, the human-origin germline having the greatest possible identity with each of the three regions V, D and J of 6F4 VH was sought. For this purpose, the nucleotide sequence of mouse 6F4  
20 VH was compared with all of the human cell line sequences present in the IMGT data base.

Human-origin germlines were identified having an sequence identity of 75.34% for the V region (IGHV1-f\*01; SEQ ID No. 46; EMBL nomenclature: Z12305), of 71.42% for the D region (IGHD1-1\*01; SEQ ID No. 47; EMBL nomenclature: X97051) and of 87.51% for the J region (IGHJ4\*01; SEQ ID No. 48, EMBL  
25 nomenclature: J00256).

For each of the regions V, D and J, the germinal lines above were selected and rearranged between them.

These alignments are presented in figure 6A for the V region, figure 6B for the  
30 D region and figure 6C for the J region.

c) *Humanized versions of 6F4 VH*

The following step in the humanization process consists of joining together the IGHV1-f\*01, IGHD1-1\*01 and IGHJ4\*01 cell line sequences and then joining the mouse 6F4 VH CDRs to the scaffold regions of these same germlines.

This stage of the process the molecular model of the mouse 6F4 Fv regions will  
5 be particularly useful in the choice of the mouse residues to preserve because they may play a role either in maintaining the molecule's three-dimensional structure (canonical structure of CDRs, VH/VL interfaces, etc.) or in binding the antigen. In the scaffold regions, each difference between mouse (6F4 VH) and human (IGHV1-f\*01, IGHD1-1\*01 and IGHJ4\*01) nucleotides will be examined very carefully.

10 For more clarity in the following, figure 7 presents the 6F4VH sequence with reference to KABAT and IMGT classifications.

As was the case with the light chain, four residues that must remain unchanged were identified.

Residue 2 (Ile) is part of Vernier zone and takes part in CDR3 structuring.

15 Residue 35 (Tyr) takes part in CDR1 anchoring according to IMGT, is part of CDR1 according to Kabat, and also takes part in the VH/VL interface and interacts with CDR3.

Residue 50 (Tyr) takes part in CDR2 anchoring according to IMGT, is part of CDR2 according to Kabat, is also part of the Vernier zone and also takes part in the  
20 VH/VL interface.

Residue 59 (Arg) takes part in CDR2 anchoring according to IMGT, is part of CDR2 according to Kabat and takes part in the VH/VL interface.

A first humanized version will be able to include three mutations at residues 61, 62 and 65, respectively (IMGT classification).

25 These three residues are located in CDR2 according to Kabat and take part in the VH/VL interface.

Residue 61 (Asn/Ala) is not directly implicated in antigen recognition. Its mutation can thus be considered.

Residue 62 (Gln/Glu) and residue 65 (Lys/Gln).

30 Secondly, two additional changes will be evaluated. The two changes relate to residues 48 and 74 (IMGT nomenclature).

Residue 48 (Ile/Met), belonging to the scaffold region, takes part in the VH/VL interface.

Residue 74 (Lys/Thr) is part of the Vernier zone and may be implicated in CDR2 structuring.

- 5        Third and last, a third series of mutations could be considered, namely a change of residues 9 (Pro/Ala) and 41 (His/Pro). The aim is thus, in a way similar to the mutations planned for 6F4 VL, to approach the human germline as closely as possible without modifying CDR anchoring.

- 10        For summary purpose only, tables 4 and 5 below list the cell lines used as well as, respectively, their amino acid and nucleotide sequence numbers.

Table 4

| GERMLINES (EMBL ref.)   | SEQ ID No. |
|-------------------------|------------|
| IGKV19-93*01 (AJ235935) | 39         |
| IGKJ1*01 (V00777)       | 40         |
| IGKV1-33*01 (M64856)    | 41         |
| IGKJ1*01 (J00242)       | 42         |
| IGHV1S135*01 (AF304556) | 43         |
| IGHD-ST4*01 (M23243)    | 44         |
| IGHJ2*01 (V00770)       | 45         |
| IGHV1-f*01 (Z12305)     | 46         |
| IGHD1-1*01 (X97051)     | 47         |
| IGHJ4*01 (J00256)       | 48         |
| IGHV1-03*01 (X62109)    | 49         |

Table 5

| GERMLINES (EMBL ref.)   | SEQ ID No. |
|-------------------------|------------|
| IGKV19-93*01 (AJ235935) | 50         |
| IGKJ1*01 (V00777)       | 51         |
| IGKV1-33*01 (M64856)    | 52         |
| IGKJ1*01 (J00242)       | 53         |
| IGHV1S135*01 (AF304556) | 54         |
| IGHD-ST4*01 (M23243)    | 55         |
| IGHJ2*01 (V00770)       | 56         |
| IGHV1-f*01 (Z12305)     | 57         |
| IGHD1-1*01 (X97051)     | 58         |
| IGHJ4*01 (J00256)       | 59         |
| IGHV1-03*01 (X62109)    | 60         |

Example 4: Purification and identification of the 6F4 antibody antigen targetPurification by immunoaffinity

The antigen target of the 6F4 antibody is purified from a membrane fraction enriched by HT-29 cells. After solubilization in a 50 mM Tris/HCl buffer, pH 7.4, containing 150 mM NaCl, Triton X-100 and IGEPAL, membrane proteins are incubated in the presence of the 6F4 antibody immobilized on sepharose beads overnight at +4 °C under gentle mixing. The 6F4-Ag complex formed on the beads is then washed with various solutions containing detergents in order to eliminate proteins adsorbed nonspecifically. The 6F4 antigen target is eluted from the 6F4-sepharose support using a

0.1 M Gly/HCl buffer, pH 2.7. The fractions collected are analyzed by SDS-PAGE electrophoresis (10% gel, non-reducing conditions) and western blot after transfer to nitrocellulose membrane (primary 6F4 antibody used at 0.5 µg/ml, detection by chemiluminescence) in order to select the fractions enriched in the antigen of interest (figures 8A and 8B). The analysis by western blot confirms the absence of the protein of interest in the un-selected fractions and washings, and a specific elution of the latter at acid pH.

The enriched fractions arising from two purifications were then analyzed by SDS-PAGE electrophoresis (10% gel) and western blot under the conditions described previously. The antigen recognized by the 6F4 antibody in the western blot had an apparent molecular weight of 35 kDa after analysis in reducing conditions (figures 9A and 9B). A difference in apparent molecular weight can be noted when electrophoresis is performed in non-reducing conditions: under these conditions, the apparent molecular weight is indeed slightly lower than that observed in reducing conditions.

15

#### Identification of the antigen target

After SDS-PAGE electrophoresis (10% gel), the proteins are stained with colloidal blue using a method compatible with mass spectrometry analysis (figure 10). The band of interest corresponding to the protein detected by western blot is cut out using a scalpel and then de-stained by incubation in a 25 mM ammonium bicarbonate solution. After reduction (DTT)/alkylation (iodoacetamide) and "in gel" hydrolysis (overnight at 37°C) of the protein by trypsin (Promega), a proteolytic enzyme that hydrolyzes proteins at the Lysine and Arginine residues and thus releases peptides having a Lysine or Arginine residue in the C-terminal position, the peptides generated are extracted using an acetonitrile/water mixture (70/30, v/v) in the presence of formic acid. These are then deposited on the MALDI target in a mixture with a matrix (alpha-cyano-4-hydroxycinnamic acid, Bruker Daltonics) and in the presence of ATFA, and then analyzed by MALDI-TOF mass spectrometry (Autoflex, Bruker Daltonics). The mass spectrum obtained is presented in figure 10. The list of the peptides deduced from this analysis is used to identify the protein by searching data banks using the Mascot search engine (Matrix Sciences).

30

The NCBI nr data bank search results, restricted to proteins of human origin, indicate that three proteins have a significant score (score > 64):

1. Crystal structure of human junctional adhesion molecule type 1  
Score = 116  
5 This protein corresponds to the extracellular domain of the F11R/JAM-A protein used for structural studies.
2. F11 receptor (*Homo sapiens*)  
Score = 116  
This protein corresponds to the precursor of protein F11R/isoform a.
- 10 3. F11 receptor isoform b (*Homo sapiens*)  
Score = 65  
This is the precursor of the isoform b of protein F11R, with two deletions of 20 amino acids with respect to isoform a.

The identified protein, by this approach, is thus called F11R or F11 receptor.  
15 This is in fact the official designation of the protein adopted when it was first described as a receptor of a so-called F11 antibody (Naik *et al.*, 1995, Biochem. J., 310, 155-162). This protein is better known today under the name of JAM-A or "junctional adhesion molecule A", and is also called JAM1, PAM-1, CD321 or antigen 106.

Among the peptides released by tryptic hydrolysis and analyzed by mass  
20 spectrometry, nine peptides have an experimental molecular weight corresponding, within 0.1 Da, to that of peptides arising from the theoretical hydrolysis of the human form of JAM-A/isoform a. These nine peptides cover 37% of the protein's primary sequence. Moreover, the theoretical molecular weight of the JAM-A precursor (~32.9 kDa) is in agreement with the apparent molecular weight determined  
25 experimentally by SDS-PAGE.

#### Confirmation of the target identified by western blot

The identification of JAM-A by a proteomic approach was then confirmed by western blot (10% SDS-PAGE gel in non-reducing conditions, 6F4 antibody at  
30 0.5 µg/ml, detection by chemiluminescence).

As shown in figure 11A, the 6F4 antibody recognizes natural JAM-A protein in the HT-29 membrane extract and in the fraction enriched by immunopurification

(apparent MW = 35 kDa), as well as the dimeric recombinant protein JAM-A/Fc (R&D Systems ref. 1103-JM, apparent MW ~120 kDa). This recognition is equivalent to that of a commercial anti-human JAM-A goat polyclonal antibody (R&D Systems, ref. AF1103) diluted to 1/1000 (figure 11B).

5

#### Example 5: Specificity of the 6F4 antibody for human JAM-A

The specificity of the 6F4 antibody was determined by western blot under the conditions described above.

Figure 12 shows that the 6F4 antibody is specific for the human form of JAM-A since it recognizes the recombinant protein hJAM-A/Fc (R&D Systems ref. 1103-JM), but recognizes neither the human forms of JAM-B and JAM-C (recombinant proteins hJAM-B/Fc and hJAM-C/Fc, R&D Systems ref. 1074-VJ and 1189-J3) nor the murine form of JAM-A (recombinant protein mJAM-A/Fc, R&D Systems ref. 1077-JM).

#### Example 6: Measurement of the affinity of the 6F4 antibody by BIAcore (surface plasmon resonance)

##### Principle

Using BIAcore, the affinity constant  $K_D$  (M) of the 6F4 antibody for the soluble protein JAM-1-Fc (extracellular domain fused with a Fc fragment of the antibody and produced in recombinant form in NS0 cells) can be calculated from the determination of the association kinetics ( $k_a$ ) (1/m.s) and the dissociation kinetics ( $k_d$ ) (1/s) according to the formula  $K_D = k_d/k_a$  (Rich and Myszka, J. Mol. Recog., 2005, 18, 431).

##### Materials and methods

*Instrument used:* BIAcore X and BIAevaluation 3.1 X software (Uppsala, SW)

##### *Reagents:*

- Murine monoclonal 6F4 antibody: 1.3 mg/ml
- Human JAM-1-Fc (ref. 1103-JM R&D Systems): 50 µg carrier-free)
- Mouse JAM-1-Fc (ref. 1077-JM R&D Systems): 50 µg carrier-free
- Running buffer: HBS-EP (BIAcore)
- Binding kit: "Amine" (BIAcore)
- Binding buffer: Acetate pH 5.0 (BIAcore)

- Capturing antibody: goat IgG Fc anti-human (= GAH, goat anti-human) (Bioscience)
- Regeneration buffer: Glycine, HCl pH 1.5 for 30 seconds (BIAcore).

#### *Discussion and conclusions*

5       The data in figure 13 show that the murine 6F4 antibody is bound to the extracellular part of the human JAM-1 protein but not to the extracellular part of the murine JAM-1 protein.

      The data in figure 14 make it possible to calculate a  $K_D$  of 22 pM of the 6F4 antibody for the human JAM-1 protein under these experimental conditions.

10       The slow dissociation kinetics indicates the involvement of a phenomenon of antibody avidity for the antigen (divalent analytical model).

#### Example 7: *In vivo* activity of the 6F4 antibody in the MCF-7 xenograft model

15       A test of the 6F4 antibody, unpurified and injected by IP route at a dose of 250 µg/mouse, demonstrates that this antibody significantly inhibits the growth of MCF-7 cells *in vivo* with inhibition percentages reaching 56% compared to mice injected with PBS (figure 15). The non-relevant 9G4 antibody used as an IgG1 control isotype is, as expected, without antitumor activity.

#### 20   Example 8: Study of the distribution of the antigen recognized by 6F4 on a panel of tumor cells

      In order to determine the potential indications for the 6F4 antibody, four types of tumors were studied by flow cytometry in terms of a membrane expression profile. The selected cell lines are MCF-7 (estrogen-related breast cancer), A549 (non-small cell  
25   lung cancer), HT29 and Colo 205 (colon cancer) and BxPC3 (pancreatic cancer). For labeling cells, a range of doses (10 µg/ml, 5 µg/ml, 1 µg/ml, 0.5 µg/ml, 0.25 µg/ml and 0.125 µg/ml) was tested.

      The results presented in figure 16 show that the 6F4 antibody recognizes an antigen significantly expressed on the surface of all cells tested. The labeling obtained is  
30   saturable, which attests to its specificity. Saturation of the sites is obtained from a concentration of 1 µg/ml of antibody, which is evidence that the 6F4 antibody's affinity for the JAM-A antigen is high.

Example 9: Humanization by CDR-grafting of the variable region of the light chain of the 6F4 antibody (6F4 VL)

- Summary of the immunogenetic analysis

|                                     |                                                                             |             |                                   |
|-------------------------------------|-----------------------------------------------------------------------------|-------------|-----------------------------------|
| Result summary:                     | Productive IGK rearranged sequence<br>(no stop codon and in frame junction) |             |                                   |
| V-GENE and allele                   | IGKV1-33*01                                                                 | score = 922 | identity = 81,36%<br>(227/279 nt) |
| J-GENE and allele                   | IGKJ1*01                                                                    | score = 140 | identity = 86,49%<br>(32/37 nt)   |
| CDR-IMGT lengths and<br>AA JUNCTION | [6,3,8]                                                                     | CLQYDNLWTF  |                                   |

5 - Detailed data for closest human V-gene identification

Closest V-REGIONS (evaluated from the V-REGION first nucleotide to the 2nd-CYS codon plus 15 nt of the CDR3-IMGT)

|                        | Score | Identity            |
|------------------------|-------|---------------------|
| M64856 IGKV1-33*01     | 922   | 81,36% (227/279 nt) |
| 10 M64855 IGKV1D-33*01 | 922   | 81,36% (227/279 nt) |
| X63398 IGKV1-27*01     | 868   | 79,21% (221/279 nt) |
| Y14865 IGKV1-NL1*01    | 841   | 78,14% (218/279 nt) |
| X72817 IGKV1D-43*01    | 841   | 78,14% (218/279 nt) |

- Detailed data for closest human J-gene identification

15

Closest J-REGIONS :

|                    | Score | Identity          |
|--------------------|-------|-------------------|
| J00242 IGKJ1*01    | 140   | 86,49% (32/37 nt) |
| AF103571 IGKJ4*02  | 122   | 81,08% (30/37 nt) |
| 20 J00242 IGKJ4*01 | 113   | 78,38% (29/37 nt) |
| Z70260 IGKJ2*02    | 104   | 75,68% (28/37 nt) |
| Z46620 IGKJ2*04    | 95    | 72,97% (27/37 nt) |

- Identification of critical residues

25 Several criteriae are involved in the definition and ranking of outside CDR critical residues. These include at least, known participation of the residue in VH/VL interface, in antigen binding or in CDR structure, the amino acid class changes between murine

and human residues, localization of the residue in the 3D structure of a variable domain etc...

21 amino acids are found different between 6F4 VL domain and the closest IGKV1-33\*01 human germline V gene, all of them being outside CDR residues. Out of these 21 residues, analysis of the above cited parameters lead to the identification of 9 most potentially contributing residues. These murine residues are K24, I39, A40, H55, T66, Q68, A69, R85 and Y87. Out of these 9 residues, 3 of them are supposed to be even more important so that they will keep their murine origin in the humanized form. These are residues I39 and H55 and T66, located at the CDR1 and CDR2 anchors, respectively. Finally, 6 amino acids will be analysed individually and/or in combination to determine whether they can be humanized or if they have to keep their murine origin. Looking to the non-involvement of the J-region in antigen binding and structuration of the V-region, it was decided to use the native human IGKJ1\*01 germline gene.

In the designed sequence of the humanized 6F4 VL domain depicted in Figure 17 :

\*, correspond to amino acids changed *de facto* to their human counterparts

1, correspond to amino acids analysed for their abilities to be humanized, the human residue being indicated below the sign

2, correspond to amino acids that remain murin in the humanized 6F4 VH domain

#### Example 10: First version of humanization by CDR-grafting of the variable region of the heavy chain of the 6F4 antibody (6F4 VH)

- Summary of the immunogenetic analysis

|                                  |                                                                             |             |                                   |
|----------------------------------|-----------------------------------------------------------------------------|-------------|-----------------------------------|
| Result summary:                  | Productive IGH rearranged sequence<br>(no stop codon and in frame junction) |             |                                   |
| V-GENE and allele                | IGHV1-f*01                                                                  | score = 796 | identity = 75,35%<br>(217/288 nt) |
| J-GENE and allele                | IGHJ4*01                                                                    | score = 181 | identity = 87,23%<br>(41/47 nt)   |
| CDR-IMGT lengths and AA JUNCTION | [8,8,9]                                                                     | CARQTDYFDYW |                                   |

D-gene strictly belongs to the CDR3 region in the VH domain. The humanization process is based on a « CDR-grafting » approach. Analysis of the closest human D-genes is not usefull in this strategy.

- Detailed data for closest human V-gene identification

- 5 Closest V-REGIONS (evaluated from the V-REGION first nucleotide to the 2nd-CYS codon)

|                      | Score | Identity            |
|----------------------|-------|---------------------|
| Z12305 IGHV1-f*01    | 796   | 75,35% (217/288 nt) |
| X62106 IGHV1-2*02    | 787   | 75,00% (216/288 nt) |
| 10 X92208 IGHV1-2*03 | 782   | 74,65% (215/288 nt) |
| Z12310 IGHV1-2*04    | 778   | 74,65% (215/288 nt) |
| M99642 IGHV1-24*01   | 760   | 73,96% (213/288 nt) |

- Detailed data for closest human J-gene identification

Closest J-REGIONS :

|                    | Score | Identity          |
|--------------------|-------|-------------------|
| 15 J00256 IGHJ4*01 | 181   | 87,23% (41/47 nt) |
| X86355 IGHJ4*02    | 172   | 85,11% (40/47 nt) |
| M25625 IGHJ4*03    | 172   | 85,11% (40/47 nt) |
| J00256 IGHJ1*01    | 138   | 74,51% (38/51 nt) |
| 20 J00256 IGHJ5*01 | 133   | 74,00% (37/50 nt) |

- Identification of critical residues

- Several criteriae are involved in the definition and ranking of outside CDR critical residues. These include at least, known participation of the residue in VH/VL interface, 25 in antigen binding or in CDR structure, the amino acid class changes between murine and human residues, localization of the residue in the 3D structure of a variable domain etc...

- 31 amino acids are found different between 6F4 VH domain and the closest IGHV1-f\*01 human germline V gene, all of them being outside CDR residues. Out of these 31 30 residues, analysis of the above cited parameters lead to the identification of 9 most potentially contributing residues. These murine residues are I2, Y40, I53, Y55, R66, N68, Q69, K72 and K82. Out of these 9 residues, 2 of them are supposed to be even more important so that they will keep their murine origin in the humanized form. These are residues Y55 and R66, located at the CDR2 anchors. Finally, 7 amino acids will be

analysed individually and/or in combination to determine whether they can be humanized or if they have to keep their murine origin.

Looking to the non-involvement of the J-region in antigen binding and structuration of the V-region, it was decided to use the native human IGHJ4\*01 germline gene.

5 In the designed sequence of the humanized 6F4 VH domain depicted in Figure 18 :

\* , correspond to amino acids changed *de facto* to their human counterparts

1, correspond to amino acids analysed for their abilities to be humanized, the human residue being indicated below the sign

2, correspond to amino acids that remain murin in the humanized 6F4 VH  
10 domain

Example 11 : Second version of humanization by CDR-grafting of the variable region of the heavy chain of the 6F4 antibody (6F4 VH)

An other way to identify human V-gene candidates for CDR-grafting was to look for  
15 human homologies at the amino acid level using IMGT/DomainGapAlign tool.

- Results of the IMGT/DomainGapAlign immunogenetic analysis are summarized hereinafter:

| Allele     | Species      | Domain | Smith-Waterman Score | Identity percentage | Overlap |
|------------|--------------|--------|----------------------|---------------------|---------|
| IGHV1-3*01 | Homo sapiens | 1      | 451                  | 64.3                | 98      |

20 - Identification of critical residues in IGHV1-03\*01 germline gene (SEQ ID No. 49, EMBL nomenclature: X62109).

The alignment of 6F4 VH domain and IGHV1-3\*01 proteic sequences is represented in figure 31.

The selection and ranking of those residues is based on differential criteriae  
25 based on the relative importance of each single position according to their structural relevance, their known structure-function relationship, the relevance of the amino acid class change if it happen and it also take advantage of the results obtained during the first humanization process.

In a first intention, all the different "out-side CDRs" amino acids have been changes for their human counterparts, except residues Y55 and R66 which both are strongly supposed to be involved in binding as CDR2-anchors assigned residues. Humanizability of those two residues will be explore at the end of the process, when all  
5 the other analyses described after will be performed. Indeed, recovery of the fully activity of the parental antibody, the 6F4 Hz2 re-humanized VH domain would have to be improved as follow; a "de-humanization" process would consist in back mutating, if necessary, these amino acids in their murine counterpart:

The first group residues, namely E1Q, K43R and K75R present a strong  
10 combination of criteria and correspond to the first positions that "de-humanization" will be assessed if looking for a benefit.

Then, residues from group 2, namely K48Q, S49R, F88Y and H90R, are chemically relevant mutations but structurally a little less supposed key residues and will be tested in a second round of experiment.

15 The six residues from the third group, are presumably more involved in an overall and/or core-oriented residues and thus supposed to be less involved in binding and thus be explored in a third round of improving, whenever necessary.

Residues from the group 4, are supposed to be the less structurally and/or amino acid class change relevant and for who "de-humanization" would be explored lately.

20 Finally, the following six residues, I2V, Y40H, I53M, N68S, K72Q and K82T, correspond to amino acids that humanization did not, at least in this initial combination, alter binding activity of the firstly humanized VH domain. "De-humanization" of these residues will be performed in a last round of improving.

D-gene strictly belongs to the CDR3 region in the VH domain. The  
25 humanization process is based on a « CDR-grafting » approach. Analysis of the closest human D-genes is not usefull in this strategy.

Looking to the non-involvement of the J-region in antigen binding and structuration of the V-region, it was decided to use the native human IGHJ4\*01 germline gene.

- Experimental data obtained for the re-humanized 6F4 antibody

In the following experiments, the re-humanization only concern the heavy chain, the light chain always corresponding to the QTY/AET humanized 6F4 VL domain as exemplified in example 9 this finally selected humanized VL domain exhibits an anti-JAM-a binding activity similar to that of the recombinant chimeric 6F4 antibody. Similarly, the re-humanized version improvement assays were performed with reference to recombinant chimeric 6F4 antibody anti-JAM-a binding activity as defined by an ELISA assay (data not showed).

10 Example 12: In vitro down-regulation of JAM-A expression by the 6F4 MAb

MCF-7, HT29 and A549 cell lines were selected to determine the effect of the 6F4 MAb on JAMA expression. Briefly cells were plated in 75 cm<sup>2</sup> flasks and incubated at 37°C, in 5% CO<sub>2</sub> atmosphere, for 24 hours, in medium supplemented with 10% Fetal Calf Serum (FCS). Then cells were washed 3 times with PBS and incubated for an additional day in serum-free medium. After this second incubation, the serum-free medium was removed and replaced by fresh serum-free medium alone or fresh serum-free medium containing either 6F4 or an IgG1 isotype control described as 9G4. After either 5 or 16 hours of incubation, cold lysis buffer (10 mM Tris HCl buffer, pH 7.5, 15% NaCl 1 M (Sigma Chemical Co.), 10% detergent mix (10 mM Tris-HCl, 10% Igepal lysis buffer) (Sigma Chemical Co.), 5% sodium deoxycholate (Sigma Chemical Co.), 1 protease inhibitor cocktail complete TM tablet (Roche) and 1% phosphatase inhibitor Cocktail Set II (Calbiochem), pH 7.5) was added and cells were scrapped on ice. The lysates were clarified by centrifugation at 4°C. Protein was quantified by BCA protein assay and 25µg of protein were loaded in each lane of a Biorad 4-12% Bis-Tris gel. Samples were heated for 5 minutes at 100°C and kept at -20°C or loaded directly on 4-12% SDS-PAGE gels and transferred to nitrocellulose membrane. Blots were first blocked with 5% BSA for all antibodies. Incubation of specific anti-JAMA primary antibody was performed for 2 hours at room temperature. Filters were washed in TBST and incubated with appropriate HRP-linked secondary antibodies for 1 hour at room temperature. Membranes were washed in TBST prior visualization of proteins with ECL (Amersham).

As shown in Figure 19, a significant down-regulation of JAM-A was observed for the 3 cell lines treated with the 6F4 MAb. MCF-7 seemed to be the most sensitive one with a complete and stable down-regulation observed as early as 5 hours post 6F4 incubation. For HT29 cells a partial but sustained down-regulation of JAM-A was also noticed. The kinetic of down-regulation was different for A549 cells as no significant effect was observed at the early incubation time while a complete inhibition occurred after 16 hours of incubation with the 6F4 MAb. As expected no significant differences were observed between untreated cells and cells incubated with the 9G4 isotype control.

10 Example 13: Effect of a single injection of 6F4 on *in vivo* tumor proliferation

To determine the *in vivo* mechanism of action of the 6F4 MAb, 7 weeks old female mice bearing estrogen pellets have been injected with MCF-7 cells. When tumors reached a volume of 80 to 100 mm<sup>3</sup>, 3 groups of mice with comparable tumors were generated. Before any injection, tumors were removed from one of these groups to check the basal proliferation of tumor cells within an untreated tumor. Mice from the 2 other groups were injected either with 1 mg of 6F4 or with the same dose of an IgG1 isotype control described as 9G4.

Six hours post injection, tumors were removed, fixed in formalin, paraffin embedded, cut into 5 µm sections and stained with an anti-Ki67 antibody to determine the level of proliferation in treated versus control tumors.

As shown in figure 20 no difference was observed between tumors removed before injection (described as T0 for time 0) and tumors treated with the isotype control 9G4. On the other hand, a significant inhibition of tumor cell proliferation was observed after a single injection, 6F4.

25 Example 14: Effect of a single injection of 6F4 on *in vivo* JAM-A expression

For this study the *in vivo* protocol is the same as the one described in *in vivo* proliferation experiments except that removed tumors were quickly frozen in liquid nitrogen for Western blot analysis. The Western blot was performed as described in the Example 13 above.

Figure 21 demonstrate that no difference in JAM-A expression was observed between untreated mice (described as T0 for Time 0) and mice injected once with the

9G4 isotype control. A significant down-regulation was noticed when mice were treated with the 6F4 MAb indicating that a potential mechanism of action involved in the *in vivo* antitumor activity of this antibody could be the down-regulation of the receptor. These results were in agreement with the one observed *in vitro* and described below in example 13.

Example 15: Comparison of the anti-tumoral activity of 6F4 and its F(ab')<sub>2</sub> fragment

As JAM-A is highly expressed by MCF-7 cells and despite the fact that 6F4 is an IgG1 (isotype known to be poorly involved in effector functions in mice), an *in vivo* comparison between 6F4 and its F(ab')<sub>2</sub> fragment has been set up in the MCF-7 model to determine a potential involvement of effector functions in the *in vivo* activity.

For that purpose, Five millions MCF7 cells were engrafted into 7 weeks old mice female bearing estrogens pellet. Five days after cells implantation, mice were treated either with 300 µg of 6F4 or with 200 µg of 6F4 F(ab')<sub>2</sub> three times per week. For the first injection, 600 µg of antibody and 400 µg of 6F4 F(ab')<sub>2</sub> were injected. Tumor volume was measured twice a week for 4 weeks.

Figure 22 showed that tumor growth in mice treated with 6F4 and 6F4 F(ab')<sub>2</sub> was significantly different from tumor growth of control mice from D3 to D27 (p≤0.03 for 6F4 and p≤0.015 for 6F4 F(ab')<sub>2</sub>). No difference was observed between 6F4 and 6F4 F(ab')<sub>2</sub> groups of mice showing that effector functions are not involved in the 6F4 activity.

Example 16: Evaluation of the expression of JAM-A on human tissue

A comparison of JAM-A expression on tumoral *versus* normal patient tissues has been performed to select tumor types overexpressing JAMA. Pairs of normal *versus* tumoral tissues from the same patient were selected for this study. In these patients normal tissues was taken near to the tumor. JAM-A expression was determined by ImmunoHistoChemistry (IHC) using tissue arrays from Supershops. Briefly, Slides were dewaxed and antigen retrieval was performed using the Dakocytomation solution S1699, at 98°C for 20 minutes. After quenching endogenous peroxidase (0.3% H<sub>2</sub>O<sub>2</sub> solution for 5 minutes) and blocking non specific sites (Ultra-V-Block ; Labvision, ref. TA-I25-UB), the primary antibody (anti-hJAM-A, AF1103 from R&Dsystem or goat

IgG isotype control from Zymed) was incubated for 1 hour at room temperature. After washes in TBS-tween, the binding of the anti-hJAM-A was revealed using the LSAB+ kit from DakoCytomation. Visualization of the complex primary Ab and LSAB+ was performed by the chromogenic reaction HRP-DAB. Slides were then counterstained by hematoxylin.

Samples of thyroid, lung and breast cancer were analysed. For thyroid samples (Figure 23), no expression was observed on normal thyroid tissue while JAM-A appeared to be strongly expressed in tumoral sections (membrane staining) from the same patient. In lung normal tissue JAM-A was expressed by pneumocytes. However, a strong membrane expression was observed in all tumoral samples (Figure 24). For breast cancer, a weak JAM-A expression, located on lobular ducts, was observed on normal breast tissue. In cancer sections, the 3 examples of carcinoma shown in Figure 25 (infiltrating duct, atypically medullary and infiltrating papillary) demonstrate that JAM-A is over expressed on breast cancer tissues.

These data suggested that thyroid, breast and lung cancers could be good targets for a JAMA therapy.

Example 17: In vivo activity of 6F4 on A431 epidermoid carcinoma xenograft in nude mice

A-431 cells were routinely cultured in DMEM (Lonza) supplemented with 10% heat inactivated Fetal Calf Serum (Sigma). Cells were split two days before engraftment so that they were in exponential phase of growth. Ten million A-431 cells were engrafted on 7 weeks old Athymic Nude mice. Five days after engraftment (D5) mice were randomised and treated i.p. with the following schemes: The control group received twice a week injections of PBS and the 6F4 treated group was injected i.p. with a loading dose of 2 mg followed by twice a week injections of 1 mg dose of antibody. Tumors were measured twice a week and tumor volumes were calculated using the formula:  $\pi/6 \cdot \text{length} \cdot \text{width} \cdot \text{height}$ . Statistical analysis was performed for each time point using a Mann-Whitney Test and SigmaStat software. Figure 26 showed that the 6F4 MAb is capable of significantly inhibiting the in vivo growth of A431 cell line ( $p < 0.009$  from day 38 to day 56).

Example 18: Evaluation of 6F4 activity on antigen presentation by antigen presenting cells (APC)

JAM proteins are expressed in a variety of tissues throughout the human body as well as on the surface of platelets, leukocytes, and erythrocytes [Naik 1995; Malergue 1998; Korneki 1990; Williams 1999; Gupta 2000] . JAM-A appears to be expressed in platelets, neutrophils, monocytes, lymphocytes, and erythrocytes [For review see Mandell 2005].

To determine whether a treatment with 6F4 could impair antigen presentation in patients an evaluation of a potential interference with Antigen Presenting Cells (APC) including macrophages and dendritic cells has been performed. In the presentation process, APC internalise antigens and degrade them to generate peptides which are associated within the cytoplasm with CMH class II molecules. Then the complex is expressed on APC membranes and presented to specific T lymphocytes responding to that stimulation by proliferation.

In the study presented below, the potential effect of 6F4 on Tetanus Toxoid presentation by human PBMC was evaluated. For that purpose, PBMC were isolated by Ficoll gradient centrifugation from blood. Cells were washed in PBS, counted and suspended in RPMI 1640 medium supplemented with 10% heat-inactivated foetal calf serum (FCS), glutamine and antibiotics at the concentration of  $0.25 \cdot 10^6$  cells/ml. 100  $\mu$ l of PBMC were seeded in each well of a 96 well plate previously filled with the antigen and the antibody to be tested (10  $\mu$ g/ml final concentration). The 9G4 Mab was used as an IgG1 isotype control and phytohemagglutinin PHA (2.5  $\mu$ g/ml final concentration), a polyclonal activator of lymphocytes, was introduced as a positive control.

Specific antigen activator Tetanus Toxoid (TT) was selected and added to PBMC at a final concentration of 100  $\mu$ g/ml. Plates were then incubated at 37 °C in an atmosphere containing 5% CO<sub>2</sub> for 96 h. Then, 0.25  $\mu$ Ci of [<sup>3</sup>H]-Thymidine is added to the wells and incubated for 24 h. After incubation the cells were harvested, the filter membrane were dried and the amount of radioactivity was counted in a scintillation counter.

Regarding to figures 27A and 28A that display the values of two independent experiments, the polyclonal activator, PHA used as a positive control of PBMC preparation is a potent inducer of lymphoproliferation, with indexes ranging between 30

and 70 depending on the donors and the experiment. In these conditions, the lymphoproliferation index was not modified whatever the antibody incubated, and 6F4 did not display any significant agonist or antagonist activity. Figures 27B and 28B that display the values of two independent experiments, showed that significant variations  
5 could occur between donors towards TT activation of lymphoproliferation. In these experiments, indexes ranged between 2 and 5 depending on the donors and the experiment. However, no interference on the antigen presentation was observed in presence of 6F4.

In conclusion, despite the significant expression of JAM-A on APC and  
10 lymphocyte, the use of an antibody directed against this target does not impair neither the non specific proliferation of lymphocyte nor the antigen presentation process.

#### Example 19: Evaluation of platelet aggregation and activation after 6F4 incubation

In order to investigate whether 6F4, which binds to human platelets, could have  
15 any biological function, two parameters were measured: platelet aggregation and serotonin release.

For this purpose, human platelets from 10 normal donors were incubated with 5 µg/ml of several antibodies to be tested.

PM6/248 (an anti- $\alpha$ IIb $\beta$ 3) have been reported to induce platelet aggregation.  
20 9G4 was used as negative isotype control.

As expected when tested on human platelets, thrombine and ADP induced aggregation. PM6/248 also induced platelet aggregation.

No platelet aggregation was measured after incubation with 6F4. The effect was comparable to the one observed with 9G4, used as positive control (figure 29).

25 In a similar way, 6F4 was not able to induce serotonin release (figure 30) whereas thrombine induced, as expected, 5-HT release.

All together, these results indicate that whereas JAM-A is expressed, no biological function is triggered on human platelet after 6F4 activation.

## CLAIMS

1. An isolated antibody, or a derived compound or functional fragment of same, capable of inhibiting the proliferation of tumor cells *in vitro* and/or *in vivo*, characterized in that it comprises a light chain comprising the following three CDRs:

CDR-L1 of the sequence SEQ ID No. 1;

CDR-L2 of the sequence SEQ ID No. 3; and

CDR-L3 of the sequence SEQ ID No. 5;

and a heavy chain comprising the following three CDRs:

CDR-H1 of the sequence SEQ ID No. 2;

CDR-H2 of the sequence SEQ ID No. 4; and

CDR-H3 of the sequence SEQ ID No. 6.

2. An isolated antibody, or a derived compound or functional fragment of same, according to claim 1, characterized in that it comprises a light chain comprising the following three CDRs as defined according to IMGT:

CDR-L1 of the sequence SEQ ID No. 1;

CDR-L2 of the sequence SEQ ID No. 3; and

CDR-L3 of the sequence SEQ ID No. 5;

and a heavy chain comprising the following three CDRs as defined according to IMGT:

CDR-H1 of the sequence SEQ ID No. 7;

CDR-H2 of the sequence SEQ ID No. 4; and

CDR-H3 of the sequence SEQ ID No. 12.

3. An antibody, or a derived compound or functional fragment of same, according to claim 1 or 2, characterized in that it comprises a light chain variable domain sequence comprising the amino acid sequence SEQ ID No. 13 and a heavy chain variable domain sequence comprising the amino acid sequence SEQ ID No. 14.

4. An antibody, or a derived compound or functional fragment of same, according to any of the claims 1 to 3, characterized in that said antibody is a murine antibody and in that it comprises a light chain of amino acid sequence SEQ ID No. 15, and a heavy chain of amino acid sequence SEQ ID No. 16.

5. An antibody, or a derived compound or functional fragment of same, according to any of the preceding claims, characterized in that said antibody is a chimeric antibody which also comprises constant regions of the light chain and the heavy chain derived from an antibody of a species heterologous to mouse.

6. A chimeric antibody, or a derived compound or functional fragment of same, according to claim 4, characterized in that said heterologous species is man.

7. A humanized antibody, or a derived compound or functional fragment of same, according to claim 6, characterized in that the constant regions of the light chain and the heavy chain derived from human antibody are the lambda or kappa and the gamma-1, gamma-2 or gamma-4 regions, respectively.

8. A humanized antibody, or a derived compound or functional fragment of same, according to claim 7, characterized in that it comprises a light chain variable domain sequence comprising the amino acid sequence SEQ ID No. 17, and a heavy chain variable domain sequence comprising the amino acid sequence SEQ ID No. 18 or 19.

9. An antibody, or a derived compound or functional fragment of same, according to any of the preceding claims, characterized in that said derived compound consists in a binding protein comprising a peptide scaffold on which at least one CDR is grafted in such a way as to preserve all or part of the paratopic recognition properties of the initial antibody.

10. An antibody, or a derived compound or functional fragment of same, according to any of the preceding claims, characterized in that said functional fragment is selected among the fragments Fv, Fab, (Fab')<sub>2</sub>, Fab\*, scFv, scFv-Fc and diabodies, or any fragment whose half-life has been increased such as pegylated fragments.

11. A murine hybridoma filed with the CNCM, Pasteur Institute, Paris, July 6, 2006, under number I-3646.

12. An antibody secreted by the hybridoma according to claim 11.

13. An antibody, or a derived compound or functional fragment of same, according to any of the preceding claims, characterized in that it is able to specifically bind to JAM-A (junction adhesion molecule-A) protein.

14. An antibody, or a derived compound or functional fragment of same, according to claim 13, characterized in that it has a  $K_d$  for the JAM-A protein between roughly 1 nM and 1 pM, more preferentially between 10 pM and 40 pM.

15. An isolated nucleic acid characterized in that it is selected among the following nucleic acids:

a) a nucleic acid, DNA or RNA, coding for an antibody, or for a derived compound or functional fragment of same, according to one of the claims 1 to 10 and 12 to 14;

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

c) a nucleic acid of at least 18 nucleotides capable of hybridizing under highly stringent conditions with at least one of the CDRs of nucleic acid sequences SEQ ID Nos. 20, 22 to 24, 26 and 27; and

d) a nucleic acid of at least 18 nucleotides capable of hybridizing under highly stringent conditions with at least the light chain of nucleic acid sequence SEQ ID No. 32 or 36 and/or the heavy chain of nucleic acid sequence SEQ ID No. 33, 37 or 38.

16. A vector composed of a nucleic acid according to claim 15.

17. A host cell comprising a vector according to claim 16.

18. A method for producing an antibody, or a derived compound or functional fragment of same, according to one of the claims 1 to 10 and 12 to 14, 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 claim 17; and

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

19. An antibody, or a derived compound or functional fragment of same, according to any of the claims 1 to 10 and 12 to 14, characterized in that it consists of a bispecific antibody and such that it comprises a second motif capable of interacting with a receptor implicated in tumor development, selected among the receptors VEGFR, VEGF, EGFR, IGF-1R, HER2neu, HGF, cMET, FGF, CXCR4 and CXCR2.

20. A composition comprising as an active ingredient a compound consisting of an antibody, or a derived compound or functional fragment of same, according to one of the claims 1 to 10, 12 to 14 and 19.

21. A composition according to claim 20, characterized in that it comprises, in addition, as a combination product for use in a simultaneous, separated or extended fashion, an antitumor antibody other than an antibody directed against JAM-A protein.

22. A composition according to claim 20 or 21, characterized in that it comprises, in addition, as a combination product for use in a simultaneous, separated or extended fashion, a cytotoxic/cytostatic agent.

23. A composition according to claim 22, characterized in that said cytotoxic/cytostatic agent is chemically bound with at least one of the elements of said composition for simultaneous use.

24. A composition according to any of the claims 20 to 23, characterized in that at least one of said antibodies, or the derived compounds or functional fragments of same, is conjugated with a cellular toxin and/or a radioisotope.

25. An antibody, or a derived compound or functional fragment of same, according to one of the claims 1 to 10, 12 to 14 and 19, or a composition according to one of the claims 20 to 24, for use as a drug.

26. An antibody, or a derived compound or functional fragment of same, according to one of the claims 1 to 10, 12 to 14 and 19, or a composition according to one of the claims 20 to 24, for the prevention or the treatment of a disease related to tumor cell proliferation.

27. The antibody or the composition according to claim 26 for cancer prevention or treatment.

28. The antibody or the composition according to claim 27, characterized in that said cancer is a cancer selected among prostate cancer, osteosarcoma, lung cancer, breast cancer, endometrial cancer, multiple myeloma, ovarian cancer, pancreatic cancer and colon cancer.

29. The antibody or the composition according to claim 27, characterized in that said cancer is a cancer selected among estrogen-related breast cancer, non-small cell lung cancer, colon cancer and pancreatic cancer.

30. Use of an antibody, or a derived compound or functional fragment of same, according to one of the claims 1 to 10, 12 to 14 and 19, or a composition according to one of the claims 20 to 24 in the preparation of a medicament for the prevention or the treatment of a disease related to tumor cell proliferation.

31. The use according to claim 30, wherein the disease is cancer.

32. The use according to claim 31, wherein said cancer is a cancer selected among prostate cancer, osteosarcoma, lung cancer, breast cancer, endometrial cancer, multiple myeloma, ovarian cancer, pancreatic cancer and colon cancer.

33. The use according to claim 31, wherein said cancer is a cancer selected among estrogen-related breast cancer, non-small cell lung cancer, colon cancer and pancreatic cancer.

## SEQUENCE LISTING

<110> Pierre Fabre Médicament  
GOETSCH Liliane  
CORVAIA Nathalie  
HAEUW Jean-François  
BES Cédric

<120> Novel antiproliferation antibodies

<130> D24958

<150> FR0610329

<151> 2006-11-24

<160> 64

<170> PatentIn version 3.3

<210> 1

<211> 6

<212> PRT

<213> mus musculus

<400> 1

Gln Asp Ile Asn Asn Tyr  
1 5

<210> 2

<211> 4

<212> PRT

<213> mus musculus

<400> 2

Thr Asp Tyr Ser  
1

<210> 3

<211> 3

<212> PRT

<213> mus musculus

<400> 3

Tyr Thr Ser  
1

<210> 4

<211> 8

<212> PRT

<213> mus musculus

<400> 4

Ile Asp Pro Tyr Asn Gly Gly Thr

1 5

<210> 5  
 <211> 8  
 <212> PRT  
 <213> mus musculus

<400> 5

Leu Gln Tyr Asp Asn Leu Trp Thr  
 1 5

<210> 6  
 <211> 7  
 <212> PRT  
 <213> mus musculus

<400> 6

Gln Thr Asp Tyr Phe Asp Tyr  
 1 5

<210> 7  
 <211> 8  
 <212> PRT  
 <213> mus musculus

<400> 7

Gly Tyr Ser Phe Thr Asp Tyr Ser  
 1 5

<210> 8  
 <211> 11  
 <212> PRT  
 <213> mus musculus

<400> 8

Lys Ala Ser Gln Asp Ile Asn Asn Tyr Ile Ala  
 1 5 10

<210> 9  
 <211> 6  
 <212> PRT  
 <213> mus musculus

<400> 9

Thr Asp Tyr Ser Met Tyr  
 1 5

<210> 10  
 <211> 7  
 <212> PRT

<213> mus musculus

<400> 10

Tyr Thr Ser Thr Leu Gln Ala  
1 5

<210> 11

<211> 17

<212> PRT

<213> mus musculus

<400> 11

Tyr Ile Asp Pro Tyr Asn Gly Gly Thr Arg Tyr Asn Gln Lys Phe Lys  
1 5 10 15  
Gly

<210> 12

<211> 9

<212> PRT

<213> mus musculus

<400> 12

Ala Arg Gln Thr Asp Tyr Phe Asp Tyr  
1 5

<210> 13

<211> 106

<212> PRT

<213> mus musculus

<400> 13

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

<210> 14

<211> 116

<212> PRT

<213> mus musculus

<400> 14

```

Glu Ile Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1      5      10      15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Asp Tyr
      20      25      30
Ser Met Tyr Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
      35      40      45
Gly Tyr Ile Asp Pro Tyr Asn Gly Gly Thr Arg Tyr Asn Gln Lys Phe
      50      55      60
Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Thr Ala Phe
65      70      75      80
Met His Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
      85      90      95
Ala Arg Gln Thr Asp Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Thr Leu
      100      105      110
Thr Val Ser Ser
      115

```

```

<210> 15
<211> 215
<212> PRT
<213> mus musculus

```

```

<400> 15

```

```

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

```

```

<210> 16
<211> 440
<212> PRT
<213> mus musculus

```

&lt;400&gt; 16

```

Glu Ile Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1      5      10      15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Asp Tyr
20      25      30
Ser Met Tyr Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
35      40      45
Gly Tyr Ile Asp Pro Tyr Asn Gly Gly Thr Arg Tyr Asn Gln Lys Phe
50      55      60
Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Thr Ala Phe
65      70      75      80
Met His Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85      90      95
Ala Arg Gln Thr Asp Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Thr Leu
100     105     110
Thr Val Ser Ser Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala
115     120     125
Pro Gly Ser Ala Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu
130     135     140
Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly
145     150     155     160
Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp
165     170     175
Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro
180     185     190
Ser Glu Thr Val Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys
195     200     205
Val Asp Lys Lys Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile
210     215     220
Cys Thr Val Pro Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro
225     230     235     240
Lys Asp Val Leu Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val
245     250     255
Val Asp Ile Ser Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val
260     265     270
Asp Asp Val Glu Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln
275     280     285
Phe Asn Ser Thr Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln
290     295     300
Asp Trp Leu Asn Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala
305     310     315     320
Phe Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro
325     330     335
Lys Ala Pro Gln Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala
340     345     350
Lys Asp Lys Val Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu
355     360     365
Asp Ile Thr Val Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr
370     375     380
Lys Asn Thr Gln Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe Val Tyr
385     390     395     400
Ser Lys Leu Asn Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe
405     410     415
Thr Cys Ser Val Leu His Glu Gly Leu His Asn His His Thr Glu Lys
420     425     430
Ser Leu Ser His Ser Pro Gly Lys
435     440

```

&lt;210&gt; 17

<211> 106  
 <212> PRT  
 <213> mus musculus

<400> 17

```

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

```

<210> 18  
 <211> 116  
 <212> PRT  
 <213> mus musculus

<400> 18

```

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1           5           10           15
Thr Val Lys Ile Ser Cys Lys Val Ser Gly Tyr Ser Phe Thr Asp Tyr
           20           25           30
Ser Met His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
           35           40           45
Gly Tyr Ile Asp Pro Tyr Asn Gly Gly Thr Arg Tyr Ala Glu Lys Phe
           50           55           60
Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Asp Thr Ala Tyr
65           70           75           80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
           85           90           95
Ala Arg Gln Thr Asp Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
           100           105           110
Thr Val Ser Ser
           115

```

<210> 19  
 <211> 116  
 <212> PRT  
 <213> mus musculus

<400> 19

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1           5           10           15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Asp Tyr
           20           25           30
Ser Met His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Met
           35           40           45
Gly Tyr Ile Asp Pro Tyr Asn Gly Gly Thr Arg Tyr Ser Gln Lys Phe

```

```

      50              55              60
Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr
65              70              75              80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
      85              90              95
Ala Arg Gln Thr Asp Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
      100              105              110
Thr Val Ser Ser
      115

```

```

<210> 20
<211> 18
<212> DNA
<213> mus musculus

```

```

<400> 20
caagacatta acaattat

```

18

```

<210> 21
<211> 12
<212> DNA
<213> mus musculus

```

```

<400> 21
actgactaca gc

```

12

```

<210> 22
<211> 9
<212> DNA
<213> mus musculus

```

```

<400> 22
tacacatct

```

9

```

<210> 23
<211> 24
<212> DNA
<213> mus musculus
<400> 23
attgatacctt acaatggtgg tact

```

24

```

<210> 24
<211> 24
<212> DNA
<213> mus musculus
<400> 24
ctacagtatg ataatctgtg gacg

```

24

```

<210> 25

```

<211> 21  
 <212> DNA  
 <213> mus musculus

<400> 25

cagacggact actttgacta c 21

<210> 26  
 <211> 24  
 <212> DNA  
 <213> mus musculus

<400> 26

ggttactcat tcaactgacta cagc 24

<210> 27  
 <211> 27  
 <212> DNA  
 <213> mus musculus

<400> 27

gcaagacaga cggactactt tgactac 27

<210> 28  
 <211> 33  
 <212> DNA  
 <213> mus musculus

<400> 28

aaggcaagcc aagacattaa caattatata gct 33

<210> 29  
 <211> 21  
 <212> DNA  
 <213> mus musculus

<400> 29

tacacatcta cattacaagc a 21

<210> 30  
 <211> 18  
 <212> DNA  
 <213> mus musculus

<400> 30

actgactaca gcatgtac 18

<210> 31  
 <211> 51

<212> DNA  
<213> mus musculus

<400> 31

tatattgata cttacaatgg tgggtactagg tacaaccaga agttcaaggg c 51

<210> 32  
<211> 318  
<212> DNA  
<213> mus musculus

<400> 32

gacatccaga tgacacagtc tccatcctca ctgtctgcat ctctgggagg caaagtcacc 60  
atcacttgca aggcaagcca agacattaac aattatatag cttggtacca acacaagcct 120  
ggaaaaggct ctaggctgct catacattac acatctacat tacaagcagg catcccatca 180  
aggttcagtg gaagtgggtc tgggagagat tattccttca gcatcagcaa cctggagcct 240  
gaagatattg gaacttatta ttgtctacag tatgataatc tgtggacgtt cgggtggaggc 300  
accaagctgg aaatcaaa 318

<210> 33  
<211> 348  
<212> DNA  
<213> mus musculus

<400> 33

gagatccagc tgcagcagtc tggacctgag ctggtgaagc ctggggcttc agtgaaggta 60  
tcctgcaagg cttctgggta ctcattcact gactacagca tgtactgggt gaagcagagc 120  
catggaaaga gocttgagtg gattggatat attgatacct acaatgggtg tactagggtac 180  
aaccagaagt tcaagggcaa ggccacattg actggttgaca agtcctccag cacagccttc 240  
atgcatttca acagcctgac atctgaggac tctgcagctc attactgtgc aagacagagc 300  
gactactttg actactgggg ccaaggcacc actctcacag tctcctca 348

<210> 34  
<211> 639  
<212> DNA  
<213> mus musculus

<400> 34

gacatccaga tgacacagtc tccatcctca ctgtctgcat ctctgggagg caaagtcacc 60  
atcacttgca aggcaagcca agacattaac aattatatag cttggtacca acacaagcct 120  
ggaaaaggct ctaggctgct catacattac acatctacat tacaagcagg catcccatca 180  
aggttcagtg gaagtgggtc tgggagagat tattccttca gcatcagcaa cctggagcct 240  
gaagatattg gaacttatta ttgtctacag tatgataatc tgtggacgtt cgggtggaggc 300  
accaagctgg aaatcaaacg ggctgatgct gcaccaactg tatccatctt cccaccatcc 360  
agtggagcag taacatctgg aggtgcctca gtcgtgtgct tcttgaacaa cttctacccc 420  
aaagacatca atgtcaagtg gaagattgat ggcagtgaac gacaaaatgg cgtcctgaac 480  
agttggactg atcaggacag caaagacagc acctacagca tgagcagcac cctcaogttg 540  
accaaggagc agtatgaacg acataacagc tatacctgtg aggccactca caagacatca 600  
acttcaccca ttgtcaagag cttcaacagg aatgagtgt 639

<210> 35  
<211> 1320  
<212> DNA

&lt;213&gt; mus musculus

&lt;400&gt; 35

|             |            |             |             |             |            |      |
|-------------|------------|-------------|-------------|-------------|------------|------|
| gagatccagc  | tgcagcagtc | tggaacctgag | ctggtgaagc  | ctggggcttc  | agtgaaggta | 60   |
| tcctgcaagg  | cttctggtta | ctcattcact  | gactacagca  | tgtactgggt  | gaagcagagc | 120  |
| catggaaaga  | gccttgagtg | gattggatat  | attgatcctt  | acaatgggtg  | tactaggtac | 180  |
| aaccagaagt  | tcaagggcaa | ggccacattg  | actgttgaca  | agtcctccag  | cacagccttc | 240  |
| atgcatctca  | acagcctgac | atctgaggac  | tctgcagtct  | attactgtgc  | aagacagacg | 300  |
| gactactttg  | actactgggg | ccaaggcacc  | actctcacag  | tctcctcagc  | caaaacaaca | 360  |
| gccccatcgg  | tctatccact | ggccccctgga | tctgctgccc  | aaactaactc  | catggtgacc | 420  |
| ctgggatgcc  | tggtcaaggg | ctatttccct  | gagccagtga  | cagtgaacctg | gaactctgga | 480  |
| tcctctgtcca | gcggtgtgca | caccttccca  | gctgtcctgc  | agtctgacct  | ctacactctg | 540  |
| agcagctcag  | tgactgtccc | ctccagcacc  | tggccccagcg | agaccgtcac  | ctgcaacggt | 600  |
| gccccaccgg  | ccagcagcac | caaggtggac  | aagaaaattg  | tgcccaggga  | ttgtggttgt | 660  |
| aagccttgca  | tatgtacagt | cccagaagta  | tcactctgtct | tcactctccc  | ccaaaagccc | 720  |
| aaggatgtgc  | tcaccattac | tctgactcct  | aaggtcacgt  | gtgttgttgt  | agacatcagc | 780  |
| aaggatgata  | ccgaggtcca | gttcagctgg  | tttgtagatg  | atgtggaggt  | gcacacagct | 840  |
| cagacgcaac  | cccgggagga | gcagttcaac  | agcactttcc  | gctcagtcag  | tgaacttccc | 900  |
| atcatgcacc  | aggactggct | caatggcaag  | gagttcaaat  | gcagggtcaa  | cagtgacgct | 960  |
| ttccctgccc  | ccatcgagaa | aacctctcc   | aaaaccaaag  | gcagaccgaa  | ggctccacag | 1020 |
| gtgtacacca  | ttccacctcc | caaggagcag  | atggccaagg  | ataaagtcag  | tctgacctgc | 1080 |
| atgataacag  | acttcttccc | tgaagacatt  | actgtggagt  | ggcagtgga   | tgggcagcca | 1140 |
| gcggagaact  | acaagaacac | tcagcccatc  | atggacacag  | atggctctta  | cttcgtctac | 1200 |
| agcaagctca  | atgtgcagaa | gagcaactgg  | gaggcaggaa  | atactttcac  | ctgctctgtg | 1260 |
| ttacatgagg  | gcctgcacaa | ccaccatact  | gagaagagcc  | tctccactc   | tcctggtaaa | 1320 |

&lt;210&gt; 36

&lt;211&gt; 318

&lt;212&gt; DNA

&lt;213&gt; mus musculus

&lt;400&gt; 36

|             |            |            |            |            |            |     |
|-------------|------------|------------|------------|------------|------------|-----|
| gacatacaga  | tgactcagag | cccatcatca | ttgagcgcgt | ctgtcgcgca | tccgggtacc | 60  |
| attacctgcc  | aggcaagtca | agatatcaac | aactatatgt | cttggtatca | acagaagccc | 120 |
| ggtaaagccc  | caaagctgct | gatacactac | acctccaccc | tggagaccgg | cgtgccttct | 180 |
| agatttttctg | gaagcgggtc | cggaaccgat | tatacgttca | caatctccag | ccttcagccc | 240 |
| gaagacatcg  | ccacatacta | ctgtctgcaa | tacgacaatc | tgtggacatt | tggccagggg | 300 |
| actaagggtg  | agatcaaa   |            |            |            |            | 318 |

&lt;210&gt; 37

&lt;211&gt; 345

&lt;212&gt; DNA

&lt;213&gt; mus musculus

&lt;400&gt; 37

|             |            |            |            |            |            |     |
|-------------|------------|------------|------------|------------|------------|-----|
| gaagtgcagc  | tggttcagag | cggcgccgag | gtaaaaccag | gggcgacggt | gaagataagc | 60  |
| tgcaagggtga | gtgggtactc | attcaccgac | tattcaatgc | actgggtcca | acaggccctt | 120 |
| ggtaaaggac  | tggagtggat | gggatacatc | gatccctaca | atggaggcac | taggtacgcc | 180 |
| gagaagtcc   | aggggagagt | cactattacc | gcagatactt | ctaccgatac | tgctcatatg | 240 |
| gaactcagca  | gtctgcggtc | cgaggacaca | gcagtctact | attgtgctcg | ccaaacagac | 300 |
| tattttgact  | attggggcca | gggaaccttg | gtgacagtgt | cctct      |            | 345 |

&lt;210&gt; 38

&lt;211&gt; 348

&lt;212&gt; DNA

<213> mus musculus

<400> 38

```

cagggtgcaat tggtagacagtc aggcgcggag gtgaagaagc ctggggctag tggttaaagtc      60
tcctgtaaaag cctccggata ttccctcact gactactcta tgcattgggt tcgccaggca      120
ccagggcagc ggctggaatg gatgggggtac attgatccct acaacggagg caccgcgatat      180
agtcagaagt tcacagggtcg ggtgacaatc acagccgata cgtccaccag caccgcctac      240
atggagttga gcagtctcag gtcagaagac acagccgtgt actattgcgc aagacagacc      300
gattatttcg actactgggg ccaaggcact ctctgaccg tctctagc      348

```

<210> 39

<211> 115

<212> PRT

<213> mus musculus

<400> 39

```

Met Arg Pro Ser Ile Gln Phe Leu Gly Leu Leu Leu Phe Trp Leu His
1           5           10           15
Gly Ala Gln Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser
          20           25           30
Ala Ser Leu Gly Gly Lys Val Thr Ile Thr Cys Lys Ala Ser Gln Asp
          35           40           45
Ile Asn Lys Tyr Ile Ala Trp Tyr Gln His Lys Pro Gly Lys Gly Pro
          50           55           60
Arg Leu Leu Ile His Tyr Thr Ser Thr Leu Gln Pro Gly Ile Pro Ser
65           70           75           80
Arg Phe Ser Gly Ser Gly Ser Gly Arg Asp Tyr Ser Phe Ser Ile Ser
          85           90           95
Asn Leu Glu Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln Tyr Asp
          100          105          110
Asn Leu Leu
          115

```

<210> 40

<211> 12

<212> PRT

<213> mus musculus

<400> 40

```

Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
1           5           10

```

<210> 41

<211> 117

<212> PRT

<213> homo sapiens

<400> 41

```

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Gln Leu Trp
1           5           10           15
Leu Ser Gly Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
          20           25           30
Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Gln Ala Ser
          35           40           45

```

Gln Asp Ile Ser Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys  
 50 55 60  
 Ala Pro Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val  
 65 70 75 80  
 Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr  
 85 90 95  
 Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln  
 100 105 110  
 Tyr Asp Asn Leu Pro  
 115

<210> 42  
 <211> 12  
 <212> PRT  
 <213> homo sapiens

<400> 42

Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
 1 5 10

<210> 43  
 <211> 98  
 <212> PRT  
 <213> mus musculus

<400> 43

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

<210> 44  
 <211> 2  
 <212> PRT  
 <213> mus musculus

<400> 44

Gln Thr  
 1

<210> 45  
 <211> 16  
 <212> PRT  
 <213> mus musculus

&lt;400&gt; 45

```

Asp Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
1           5           10           15

```

&lt;210&gt; 46

&lt;211&gt; 98

&lt;212&gt; PRT

&lt;213&gt; homo sapiens

&lt;400&gt; 46

```

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

```

&lt;210&gt; 47

&lt;211&gt; 121

&lt;212&gt; PRT

&lt;213&gt; homo sapiens

&lt;400&gt; 47

```

Met Ser Val Ser Phe Leu Ile Phe Leu Pro Val Leu Gly Leu Pro Trp
1           5           10           15
Gly Val Leu Ser Gln Val Gln Leu Gln Gln Ser Gly Pro Gly Leu Val
          20           25           30
Lys Pro Ser Gln Thr Leu Ser Leu Thr Cys Ala Ile Ser Gly Asp Ser
          35           40           45
Val Ser Ser Asn Ser Ala Ala Trp Asn Trp Ile Arg Gln Ser Pro Ser
          50           55           60
Arg Gly Leu Glu Trp Leu Gly Arg Thr Tyr Tyr Arg Ser Lys Trp Tyr
65           70           75           80
Asn Asp Tyr Ala Val Ser Val Lys Ser Arg Ile Thr Ile Asn Pro Asp
          85           90           95
Thr Ser Lys Asn Gln Phe Ser Leu Gln Leu Asn Ser Val Thr Pro Glu
          100          105          110
Asp Thr Ala Val Tyr Tyr Cys Ala Arg
          115          120

```

&lt;210&gt; 48

&lt;211&gt; 15

&lt;212&gt; PRT

&lt;213&gt; homo sapiens

&lt;400&gt; 48

Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser  
 1 5 10 15

<210> 49  
 <211> 97  
 <212> PRT  
 <213> homo sapiens

<400> 49

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

<210> 50  
 <211> 643  
 <212> DNA  
 <213> mus musculus

<400> 50

cagatgaagc tgatttgcac gtgctgagat catattctac tgccccagag atttaataat 60  
 ctgatcatac acaactccaac agtcattctt ggtcaggaga cgttgtagaa atgagaccgt 120  
 ctattoagtt cctggggctc ttgttgttct ggcttcacgg taaggagttt aacattgaat 180  
 atgctaaaaa gagtatgtga tcaggaattt ctggctcctc agaaaaatct tctttgaata 240  
 taattaattt catagggatt tgtgttcttt ttaattatag gtgctcagtg tgacatccag 300  
 atgacacagt ctccatcctc actgtctgca tctctgggag gcaaagtcac catcacttgc 360  
 aaggcaagcc aagacattaa caagtatata gcttggtacc aacacaagcc tggaaaaggt 420  
 cctaggctgc tcatacatca cacatctaca ttacagccag gcatcccatc aaggttcagt 480  
 ggaagtgggt ctgggagaga ttattccttc agcatcagca acctggagcc tgaagatatt 540  
 gcaacttatt attgtctaca gtatgataat cttctacceca cagtataca aatcataaca 600  
 aaaaccaccc agggaagcag aagtgaagg ctaggttgcc cac 643

<210> 51  
 <211> 39  
 <212> DNA  
 <213> mus musculus

<400> 51  
 tggacgttcg gtggaggcac caagctggaa atcaaacgt 39

<210> 52  
 <211> 667  
 <212> DNA  
 <213> homo sapiens

&lt;400&gt; 52

|            |            |            |             |             |             |     |
|------------|------------|------------|-------------|-------------|-------------|-----|
| ctgcagctgt | gccagcctg  | ccctatcccc | tgctgatttg  | catgttcgca  | gagcacagcc  | 60  |
| ccctgccctg | aagcattatt | aataggcttg | tcgcaccctg  | tgcaggagtc  | agtcccaacc  | 120 |
| aggacacagc | atggacatga | gggtccctgc | tcagctccctg | gggctccctgc | agctctggct  | 180 |
| ctcaggtaa  | gaaggataac | actaggaatt | ttctcagcca  | gtgtgctcag  | tacagcctgg  | 240 |
| ctcttgatgg | aagccttcct | ataatatgac | taatagtatg  | aatattttgtg | tttatgtttc  | 300 |
| taatgcgagg | tgccagatgt | gacatccaga | tgacccagtc  | tccatcctcc  | ctgtctgcat  | 360 |
| ctgtaggaga | cagagtcacc | atcacttgcc | aggcagagtc  | ggacattagc  | aactatttaa  | 420 |
| attggtatca | gcagaaacca | gggaaagccc | ctaagctcct  | gatctacgat  | gcattccaatt | 480 |
| tggaaacagg | ggccccatca | aggttcagtg | gaagtggatc  | tgggacagat  | tttactttca  | 540 |
| ccatcagcag | cctgcagcct | gaagatattg | caacatatta  | ctgtcaacag  | tatgataatc  | 600 |
| tcctccccac | agtgtaacaa | gtcataacat | aaatcaccca  | ggggagcaga  | tgcgtgaggc  | 660 |
| tcagctg    |            |            |             |             |             | 667 |

&lt;210&gt; 53

&lt;211&gt; 37

&lt;212&gt; DNA

&lt;213&gt; homo sapiens

&lt;400&gt; 53

|            |            |            |         |    |
|------------|------------|------------|---------|----|
| tggacgttcg | gccaaaggac | caaggtggaa | atcaaac | 37 |
|------------|------------|------------|---------|----|

&lt;210&gt; 54

&lt;211&gt; 294

&lt;212&gt; DNA

&lt;213&gt; mus musculus

&lt;400&gt; 54

|             |            |            |            |            |            |     |
|-------------|------------|------------|------------|------------|------------|-----|
| gagatccagc  | tgcagcagtc | tggacctgag | ctggtgaagc | ctggggcttc | agtgaaggta | 60  |
| tcctgcaaagg | cttctggtta | ctcattcact | gactacaaca | tgtactgggt | gaagcagagc | 120 |
| catggaaaga  | gccttgagtg | gattggatat | attgatcctt | acaatgggtg | tactagctac | 180 |
| aaccagaagt  | tcaagggcaa | ggccacattg | actgttgaca | agtcctccag | cacagccttc | 240 |
| atgcattctca | acagcctgac | atctgaggac | tctgcagtct | attactgtgc | aaga       | 294 |

&lt;210&gt; 55

&lt;211&gt; 163

&lt;212&gt; DNA

&lt;213&gt; mus musculus

&lt;400&gt; 55

|            |            |            |            |            |            |     |
|------------|------------|------------|------------|------------|------------|-----|
| aagcttgccc | aggaaccact | agtgtctaca | cagctctgcc | cacaggggaa | acctaaccat | 60  |
| gootgcccc  | tactcagcag | gaaggctctg | aagctctgag | aggattttga | acaagttact | 120 |
| gtcacagtga | gacagctcgg | gctaccatgt | aagaaaagct | caa        |            | 163 |

&lt;210&gt; 56

&lt;211&gt; 45

&lt;212&gt; DNA

&lt;213&gt; mus musculus

&lt;400&gt; 56

|             |            |            |            |       |    |
|-------------|------------|------------|------------|-------|----|
| tacttttgact | actggggcca | aggcaccact | ctcacagtct | octca | 45 |
|-------------|------------|------------|------------|-------|----|

<210> 57  
 <211> 294  
 <212> DNA  
 <213> homo sapiens

<400> 57

```
gagggtccagc  tggtagagtc  tggggctgag  gtgaagaagc  ctgggggctac  agtgaaaatc      60
tccctgcaagc  tttotggata  caccttcacc  gactactaca  tgcactgggt  gcaacaggcc     120
cctggaaaag  ggcttgagtg  gatgggactt  gttgatcctg  aagatgggtg  aacaatatatac   180
gcagagaagt  tccagggcag  agtcaccata  accgcggaca  cgtctacaga  cacagcctac     240
atggagctga  gcagcctgag  atctgaggac  acggccgtgt  attactgtgc  aaca            294
```

<210> 58  
 <211> 17  
 <212> DNA  
 <213> homo sapiens

<400> 58

```
ggtacaactg  gaacgac      17
```

<210> 59  
 <211> 46  
 <212> DNA  
 <213> homo sapiens

<400> 59

```
tactttgact  actggggcca  aggaacctg  gtcacctgt  cctcag      46
```

<210> 60  
 <211> 291  
 <212> DNA  
 <213> homo sapiens

<400> 60

```
ggggcctcag  tgaaggtttc  ctgcaaggct  tctggataca  ccttcagggt  ccagcttggt      60
cagtcctggg  ctgaggtgaa  gaagcctact  agctatgcta  tgcattgggt  gcgccaggcc     120
cccggacaaa  ggcttgagtg  gatgggatgg  atcaacgctg  gcaatggtaa  cacaaaatat     180
tcacagaagt  tccagggcag  agtcaccatt  accagggaca  catccgcgag  cacagcctac     240
atggagctga  gcagcctgag  atctgaagac  acggctgtgt  attactgtgc  g                291
```

<210> 61  
 <211> 299  
 <212> PRT  
 <213> homo sapiens

<400> 61

```
Met Gly Thr Lys Ala Gln Val Glu Arg Lys Leu Leu Cys Leu Phe Ile
1           5           10          15
Leu Ala Ile Leu Leu Cys Ser Leu Ala Leu Gly Ser Val Thr Val His
```

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

&lt;210&gt; 62

&lt;211&gt; 897

&lt;212&gt; DNA

&lt;213&gt; homo sapiens

&lt;400&gt; 62

|                                                                    |     |
|--------------------------------------------------------------------|-----|
| atggggacaa aggcgcaagt cgagaggaaa ctgttgtgcc tcttcatatt ggcgatcctg  | 60  |
| ttgtgctccc tggcattggg cagtgttaca gtgcactctt ctgaacctga agtcagaatt  | 120 |
| cctgagaata atcctgtgaa gttgtcctgt gcctactcgg gcttttcttc tcccgtgtg   | 180 |
| gagtggaaagt ttgaccaagg agacaccacc agactcgttt gctataataa caagatcaca | 240 |
| gcttctatg aggacgggt gaccttcttg ccaactggta tcaccttcaa gtcogtgaca    | 300 |
| cggaagaca ctgggacata cacttgtatg gtctctgagg aaggcggcaa cagctatggg   | 360 |
| gaggtcaagg tcaagctcat cgtgcttgtg cctccatcca agcctacagt taacatcccc  | 420 |
| tcctctgcca ccattgggaa ccgggcagtg ctgacatgct cagaacaaga tggttcccca  | 480 |
| ccttctgaat acacctgggt caaagatggg atagtgatgc ctacgaatcc caaaagcacc  | 540 |
| cgtgccttca gcaactcttc ctatgtcctg aatcccacaa caggagagct ggtctttgat  | 600 |
| cccctgtcag cctctgatac tggagaatac agctgtgagg cacggaatgg gtatgggaca  | 660 |
| cccatgaact caaatgctgt gcgcatggaa gctgtggagc ggaatgtggg ggtcatcgtg  | 720 |
| gcagccgtcc tltgaacctt gattctcctg ggaatcttgg tttttggcat ctggtttgcc  | 780 |
| tatagccgag gccactttga cagaacaaag aaagggactt cgagtaagaa ggtgatttac  | 840 |
| agccagccta gtgccgaag tgaaggagaa ttcaaacaga cctcgtcatt cctggtg      | 897 |

<210> 63  
 <211> 259  
 <212> PRT  
 <213> homo sapiens

<400> 63

```

Met Gly Thr Lys Ala Gln Val Glu Arg Lys Leu Leu Cys Leu Phe Ile
1      5      10      15
Leu Ala Ile Leu Pro Glu Asn Asn Pro Val Lys Leu Ser Cys Ala Tyr
      20      25      30
Ser Gly Phe Ser Ser Pro Arg Ala Ala Ser Tyr Glu Asp Arg Val Thr
      35      40      45
Phe Leu Pro Thr Gly Ile Thr Phe Lys Ser Val Thr Arg Glu Asp Thr
      50      55      60
Gly Thr Tyr Thr Cys Met Val Ser Glu Glu Gly Gly Asn Ser Tyr Gly
65      70      75      80
Glu Val Lys Val Lys Leu Ile Val Leu Val Pro Pro Ser Lys Pro Thr
      85      90      95
Val Asn Ile Pro Ser Ser Ala Thr Ile Gly Asn Arg Ala Val Leu Thr
      100     105     110
Cys Ser Glu Gln Asp Gly Ser Pro Pro Ser Glu Tyr Thr Trp Phe Lys
      115     120     125
Asp Gly Ile Val Met Pro Thr Asn Pro Lys Ser Thr Arg Ala Phe Ser
130     135     140
Asn Ser Ser Tyr Val Leu Asn Pro Thr Thr Gly Glu Leu Val Phe Asp
145     150     155     160
Pro Leu Ser Ala Ser Asp Thr Gly Glu Tyr Ser Cys Glu Ala Arg Asn
      165     170     175
Gly Tyr Gly Thr Pro Met Thr Ser Asn Ala Val Arg Met Glu Ala Val
      180     185     190
Glu Arg Asn Val Gly Val Ile Val Ala Ala Val Leu Val Thr Leu Ile
      195     200     205
Leu Leu Gly Ile Leu Val Phe Gly Ile Trp Phe Ala Tyr Ser Arg Gly
210     215     220
His Phe Asp Arg Thr Lys Lys Gly Thr Ser Ser Lys Lys Val Ile Tyr
225     230     235     240
Ser Gln Pro Ser Ala Arg Ser Glu Gly Glu Phe Lys Gln Thr Ser Ser
      245     250     255
Phe Leu Val

```

<210> 64  
 <211> 777  
 <212> DNA  
 <213> homo sapiens

<400> 64

```

atggggacaa aggcgcaagt cgagaggaaa ctgtttgtgcc tcttcatatt ggcgatcctt      60
cctgagaata atcctgtgaa gttgtcctgt gcctactcgg gctttttcttc tccccgtgca      120
gcttctatag aggaccgggt gaccttcttg ccaactggta tcaccttcaa gtccgtgaca      180
cgggaagaca ctgggacata cacttgtatg gtctctgagg aaggcggcaa cagctatggg      240
gaggtcaagg tcaagctcat cgtgcttggt cctccatcca agcctacagt taacatcccc      300
tcctctgcca ccattgggaa ccgggcagtg ctgacatgct cagaacaaga tggttcccca      360
ccttctgaat acacctggtt caaagatggg atagtgatgc ctacgaatcc caaaagcacc      420
cgtgccttca gcaactcttc ctatgtcctg aatcccacaa caggagagct ggtctttgat      480
cccctgtcag cctctgatac tggagaatac agctgtgagg caccgaatgg gtatgggaca      540
cccatgactt caaatgctgt gcgcatggaa gctgtggagc ggaatgtggg ggtcatcggt      600
gcagcgcgtc ttgtaacctt gattctcctg ggaatcttg tttttggcat ctggtttgcc      660

```

```
tatagccgag gccactttga cagaacaaag aaagggactt cgagtaagaa ggtgatttac 720
agccagccta gtgcccgaag tgaaggagaa ttcaaacaga cctcgtcatt cctggtg 777
```