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(54) Title: NOVEL ANTIPROLIFERATION ANTIBODIES

(57) Abstract: The present invention relates to novel isolated antibodies, or the derived compounds or functional fragments of same, capable of inhibiting the proliferation of tumor cells *in vitro* and/or *in vivo*, said antibodies having been obtained by functional screening. More particularly, the present invention relates to the 6F4 antibody, specific to the JAM-A protein, as well as its use for the treatment of cancer. Pharmaceutical compositions composed of such antibodies are also covered.

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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 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 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.

Reference to any prior art in the specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in Australia or any other jurisdiction or that this prior art could reasonably be expected to be ascertained, understood and regarded as relevant by a person skilled in the art.

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 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.

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 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.

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 a first aspect, the invention relates to an isolated antibody, or functional fragment of same, capable of inhibiting the proliferation of tumor cells *in vitro* and/or *in vivo*; said antibody, or functional fragment of same, comprising the complementarity-determining regions (CDRs) of sequences SEQ ID Nos. 1, 2, 3, 4, 5 and 6.

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

A "derived compound" 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.

As used herein, except where the context requires otherwise the term 'comprise' and variations of the term, such as 'comprising', 'comprises' and 'comprised', are not intended to exclude other additives, components, integers or steps.

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.

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

homogeneous antibody, a prokaryotic host cell transfected with a DNA molecule coding for the homogeneous antibody, etc.) and is generally characterized by heavy chains of one and only one class and subclass, and light chains of only one type. Monoclonal antibodies are highly specific and are directed against a single antigen. In addition, in contrast with preparations of polyclonal antibodies which typically include various antibodies directed against various determinants, or epitopes, each monoclonal antibody is directed against a single epitope of the antigen.

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

More particularly, according to a preferred embodiment of the invention, the antibody, or its derived compounds or functional fragments, is characterized in that it comprises a light chain comprising at least one CDR chosen among the CDRs of amino acid sequences SEQ ID No. 1, 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 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 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-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.

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.

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.

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.

In a preferred manner, the antibodies of theinvention, 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.

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

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

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

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

The IMGT unique numbering has been defined to compare the variable domains whatever the antigen receptor, the chain type, or the species [Lefranc M.-P., Immunology Today 18, 509 (1997) / Lefranc M.-P., The Immunologist, 7, 132-136 (1999) / Lefranc, M.-P., Pommié, C., Ruiz, M., Giudicelli, V., Foulquier, E., Truong, L., Thouvenin-Contet, V. and Lefranc, Dev. Comp. Immunol., 27, 55-77 (2003)]. In the IMGT unique numbering, the conserved amino acids always have the same position, for instance cystein 23 (1st-CYS), tryptophan 41 (CONSERVED-TRP), hydrophobic amino acid 89, cystein 104 (2nd-CYS), phenylalanine or tryptophan 118 (J-PHE or J-TRP). The IMGT unique numbering provides a standardized delimitation of the

framework regions (FR1-IMGT: positions 1 to 26, FR2-IMGT: 39 to 55, FR3-IMGT: 66 to 104 and FR4-IMGT: 118 to 128) and of the complementarity determining regions: CDR1-IMGT: 27 to 38, CDR2-IMGT: 56 to 65 and CDR3-IMGT: 105 to 117. As gaps represent unoccupied positions, the CDR-IMGT lengths (shown between brackets and separated by dots, e.g. [8.8.13]) become crucial information. The IMGT unique numbering is used in 2D graphical representations, designated as IMGT Colliers de Perles [Ruiz, M. and Lefranc, M.-P., Immunogenetics, 53, 857-883 (2002) / Kaas, Q. and Lefranc, M.-P., Current Bioinformatics, 2, 21-30 (2007)], and in 3D structures in IMGT/3Dstructure-DB [Kaas, Q., Ruiz, M. and Lefranc, M.-P., T cell receptor and MHC structural data. Nucl. Acids. Res., 32, D208-D210 (2004)].

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

For more clarity, it must be understood that in the following description, and more particularly in 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 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 and IMGT numbering systems and of SEQ ID No. 8 (KAS**QDINNY**IA) 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 (**YTS**TLQA) in the kabat numbering system.

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 (GYSF**TDYS**) in the IMGT numbering system and of SEQ ID No. 9 (**TDYS**MY) in the kabat numbering system.

The CDR-H2 consists of SEQ ID No. 4 (**IDPYNGGT**) in the common and IMGT numbering systems and of SEQ ID No. 11 (Y**IDPYNGGT**RYNQKFKG) 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 (AR**QTDYFDY**) in the IMGT numbering system.

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

The percentage identity between two nucleic acid or amino acid sequences is determined by comparing the two optimally-aligned sequences in which the nucleic acid or amino acid sequence to compare can have additions or deletions compared to the reference sequence for optimal alignment between the two sequences. Percentage identity is calculated by determining the number of positions at which the amino acid nucleotide or residue is identical between the two sequences, 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; 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:
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
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 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
- 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;
- 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, NMR spectroscopy or any other technique known to a person skilled in the art);
- small size;
- few or no post-transcriptional modifications; and/or
- easy to produce, express and purify.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Such functional fragments of the antibody according to the invention consist, for example, of the fragments Fv, scFv (sc=simple chain), Fab, $F(ab')_2$, Fab', scFv-Fc or diabodies, or any fragment whose half-life has been increased by chemical modification, such as the addition of polyalkylene glycol such as polyethylene glycol

(PEGylation) (PEGylated fragments are referred to as Fv-PEG, scFv-PEG, Fab-PEG, $F(ab')_2$-PEG and Fab'-PEG), or by incorporation in a liposome, microspheres or PLGA, said fragments possessing at least one of the characteristic CDRs of the invention which is notably capable of exerting in a general manner activity, even partial, of the antibody from which it arises.

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

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

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

The invention also targets the original murine antibody, namely an antibody according to the invention, or its derived compounds or functional fragments, 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 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.

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.

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

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

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

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

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, Artherioscler. 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 σ1 (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 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 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.

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).

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;

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

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.

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

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

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

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

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

a polynucleotide of defined size can be adapted by a person skilled in the art for longer or shorter oligonucleotides, according to the procedures described in Sambrook, *et al.* (Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory; 3rd edition, 2001).

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

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

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

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

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

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

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

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

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

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

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

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

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

According to still another aspect, the present invention relates to an antibody as described above, characterized in that it is, in addition, capable of specifically binding to a human 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 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 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 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.

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 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) 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, 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; antiEpCAM antibodies such as edrecolomab, Ch 17-1A or IGN–101; antiCTP21 or 16 antibodies such as Xactin; antiDNA-Ag antibodies such as ^{131}I-Cotara TNT-1; antiMUC1 antibodies such as pemtumomab or R1150; antiMUC18 antibodies such as ABX-MA1; antiGD3 antibodies such as mitumomab; antiECA antibodies such as CeaVac or labetuzumab; antiCA125 antibodies such as OvaRex; antiHLA-DR antibodies such as apolizumab; antiCTLA4 antibodies such as MDX-010; antiPSMA antibodies such as MDX-070, ^{111}In & ^{90}Y-J591, ^{177}Lu J591, J591-DM1; antiLewis Y antibodies such as IGN311; antiangiogenesis antibodies such as AS1405 and 90YmuBC1; antiTrail-R1 antibodies such as TRAIL R1mAb or TRAIL R2mAb.

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

engineering techniques that make it possible to force heterodimerization and thus facilitate the purification of the antibody sought (Merchand *et al.*, 1998, Nature Biotech., 16:677-681).

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

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

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

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

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

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

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 inhibiter of the tyrosine kinase activity of receptors such as IGF-IR, EGFR, HER2/neu, cMET and VEGFR.

In another preferred embodiment, said inhibiter 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).

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.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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 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 method comprises the following steps:

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

b) demonstrating the antigen-antibody complex possibly formed.

Thus, the present invention also comprises the kits or accessories for the implementation of a method as described (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;

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

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

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

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

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

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

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

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

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

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

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

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).

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 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.

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: ka = $(1.38 \pm 0.001)*10^5$ $M^{-1}s^{-1}$; kd = $(0.25 \pm 1.58)*10^{-6}$ s^{-1}; Rmax (global fitting) = 371 RU; κ^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 $F(ab')_2$ 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 lyphoproliferation 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 lyphoproliferation 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+/- sd.

Figure 30 represents the serotonine release on 10 human normal donors. Results are expected as mean+/- 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 $5x10^6$ MCF-7 cells from ATCC. After a final booster injection of 10^7 MCF-7 cells, cells from lymph nods 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 $5x10^3$ cells/well in 100 µl of hybridoma medium without fetal calf serum. The plates are incubated for 24 hours at 37°C under an atmosphere of 5% CO_2. After 24 hours, 50 µl 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 µl 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 µl of hybridoma culture medium.

After roughly 52 hours of culture, each well is supplemented by 0.25 μCi 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 ≥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.

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).

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*

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.

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.

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.

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 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 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.

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 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 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 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 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.

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.

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 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).

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).

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.

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.

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 target

Purification 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.

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).

The NCBInr 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
   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.
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. 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 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 experimentally by SDS–PAGE.

<u>Confirmation of the target identified by western blot</u>

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 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).

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

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.

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

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.

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

- 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)
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

Closest J-REGIONs :

	Score	Identity
J00242 IGKJ1*01	140	86,49% (32/37 nt)
AF103571 IGKJ4*02	122	81,08% (30/37 nt)
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

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 (no stop codon and in frame junction)		
V-GENE and allele	IGHV1-f*01	score = 796	identity = 75,35% (217/288 nt)
J-GENE and allele	IGHJ4*01	score = 181	identity = 87,23% (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

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)
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
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)
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, 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 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.

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 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 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

- 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 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 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 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.

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.

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 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).

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^2 flasks and incubated at 37°C, in 5% $C0_2$ atmosphere, for 24 hours, in medium supplemented with 10% Fecal 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.

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^3, 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-Ki67antibody 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.

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')$_2$ 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')2 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')$_2$ three times per week. For the first injection, 600 µg of antibody and 400 µg of 6F4 F(ab')$_2$ 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')$_2$ was significantly different from tumor growth of control mice from D3 to D27 ($p \leq 0.03$ for 6F4 and $p \leq 0.015$ for 6F4 F(ab')$_2$). No difference was observed between 6F4 and 6F4 F(ab')$_2$ 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 Superships. 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_2O_2 solution for 5 minutes) and blocking non specific sites (Ultra-V-Block ; Labvision, ref. TA-125-UB), the primary antibody (anti-hJAM-A, AF1103 from R&Dsytem 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 perfomed 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 expresses 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-431cells 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. Tumor were measured twice a week and tumor volumes were calculated using the formula: π/6.length.width.height. Statistical analysis were 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 $0.25.10^6$ cells/ml. 100 µl of PBMC were seeded in each well of a 96 well plate previously filled with the antigen and the antibody to be tested 10 µg/ml final concentration). The 9G4 Mab was used as an IgG1 isotype control and phytohemagglutinin PHA (2.5 µg/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 µg/ml. Plates were then incubated at 37 °C in an atmosphere containing 5% CO_2 for 96 h. Then, 0.25 µCi of [^{3}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 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 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 any biological function, two parameters were measured: platelet aggregation and serotonine 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. 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).

In a similar way, 6F4 was not able to induce serotonine 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.

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An

- isolated antibody, or

- a functional fragment of same capable of inhibiting the proliferation of tumor cells *in vitro* and/or *in vivo*,

characterized in that it comprises the CDRs of sequences comprising SEQ ID Nos. 1, 2, 3, 4, 5 and 6.

2. An antibody, or a functional fragment of same, according to claim 1, characterized such that it consists of a monoclonal antibody.

3. An antibody, or a functional fragment of same, according to claim 1 or claim 2 , characterized in that it comprises a heavy chain comprising the following three CDRs, respectively CDR-H1, CDR-H2 and CDR-H3, wherein:

- CDR-H1 comprises the sequence SEQ ID No. 2, 7 or 9;

- CDR-H2 comprises the sequences SEQ ID No. 4 or 11; and

- CDR-H3 comprises the sequences SEQ ID No. 6 or 12.

4. An antibody, or a functional fragment of same, according to any one of the preceding claims, characterized in that it comprises a heavy chain comprising, as defined according to the IMGT numbering system, 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.

5. An antibody, or a functional fragment of same, according to any one of the preceding claims, characterized in that it comprises 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;

- CDR-L2 comprises the sequence SEQ ID No. 3 or 10; and

- CDR-L3 comprises the sequence SEQ ID No. 5.

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6. An antibody, or a functional fragment of same, according to any one of the preceding claims, characterized in that it comprises a light chain comprising, as defined according to the IMGT numbering system, 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.

7. An antibody, or a functional fragment of same, according to any one of the preceding claims, characterized in that it comprises, according to the IMGT number system, 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. 7;
- CDR-H2 of the sequence SEQ ID No. 4; and
- CDR-H3 of the sequence SEQ ID No. 12.

8. An antibody, or a functional fragment of same, according to any one of the preceding claims, 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.

9. A humanized antibody, or a functional fragment of same, according to any one of claims 1 to 12, 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.

10. An antibody, or a functional fragment of same, according to any one of the preceding claims, characterized in that said functional fragment is selected among the fragments Fv, Fab, $(Fab')_2$, Fab', scFv, scFv-Fc and diabodies, or any fragment whose half-life has been increased such as pegylated fragments.

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11. An antibody, or a functional fragment of same, according to any one of claims 1 to 8, characterized in that said antibody is a murine antibody which comprises a light chain of amino acid sequence SEQ ID No. 15, and a heavy chain of amino acid sequence SEQ ID No. 16.

12. An antibody, or a functional fragment of same, according to any one of claims 1 to 8, 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.

13. A chimeric antibody, or a functional fragment of same, according to claim 12, characterized in that said heterologous species is man.

14. A humanized antibody, or a functional fragment of same, according to claim 13, 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 orgamma-4 regions, respectively.

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

16. An antibody secreted by the hybridoma according to claim 15.

17. An antibody, or a functional fragment of same, according to any one of claims 1 to 14 and 16, characterized in that it is able to specifically bind to JAM-A (junction adhesion molecule-A) protein.

18. An antibody, or a functional fragment of same, according to claim 17, characterized in that it has a Kd for the JAM-A protein between about 1 nM and 1 pM.

19. An antibody according to claim 18 characterised in that it has a Kd for the JAM-A protein between 10 pM and 40 pM.

20. 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 functional fragment of same, according to any one of claims 1 to 14 and 16 to 19;

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

21. A vector composed of a nucleic acid according to claim 20.

22. A host cell comprising a vector according to claim 21.

23. A transgenic animal, except for man, comprising a cell transformed by a vector according to claim 22.

24. A method for producing an antibody, or a functional fragment of same, according to any one of claims 1 to 14 and 16 to 19, 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 22; and

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

25. An antibody, or a functional fragment of same, according to any one of claims 1 to 14, 16 to 19, for use as a drug.

26. A composition comprising as an active ingredient a compound consisting of an antibody, or a functional fragment of same, according to any one of claims 1 to 14, 16 to 19, and 25.

27. A composition according to claim 26, characterized in that it comprises, in addition, as a combination product for use in a simultaneous, separated or extended fashion, an antitumor antibody other that an antibody directed against JAM-A protein.

28. A composition according to claims 26 or 27, characterized in that it comprises, in addition, as a combination product for use in a simultaneous, separated or extended fashion, a cytotoxic/cytostatic agent.

29. A composition according to claim 28, characterized in that said cytotoxic/cytostatic agent is chemically bound with at least one of the elements of said composition for simultaneous use.

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30. A composition according to any one of claims 26 to 29, 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.

31. A composition according to one of the claims 26 to 30, for use as a drug.

32. The use of an antibody, or a functional fragment of same, according to any one of claims 1 to 14, 16 to 19, and 25, and/or of a composition according to any one of claims 26 to 30, for the preparation of a drug for the prevention or the treatment of a disease related to tumor cell proliferation.

33. The use according to claim 32, for the preparation of a drug for cancer prevention or treatment.

34. The use according to claim 33, 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.

35. The use according to claim 34, characterized in that said cancer is a cancer selected among estrogen-related breast cancer, non-small cell lung cancer, colon cancer and pancreatic cancer.

36. A method of treatment of a disease related to tumour cell proliferation comprising administering an antibody or a functional fragment of same, according to any one of claims 1 to 14, 16 to 18 and 25 and/or a composition according to any one of claims 26 to 30, to a patient in need of treatment.

37. An antibody according to claim 1 substantially as hereinbefore described.

6F4 antibody

Heavy chain (variable domain):

Nucleotide sequence:

GAGATCCAGCTGCAGCAGTCTGGACCTGAGCTGGTGAAGCCTGGGGCTTCAGTGAAGGTATC

CTGCAAGGCTTCTGGTTACTCATTC**ACTGACTACAGCATGTAC**TGGGTGAAGCAGAGCCATG
CDR 1

GAAAGAGCCTTGAGTGGATTGGA**TATATTGATCCTTACAATGGTGGTACTAGGTACAACCAG**
CDR 2

AAGTTCAAGGGCAAGGCCACATTGACTGTTGACAAGTCCTCCAGCACAGCCTTCATGCATCT

CAACAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGA**CAGACGGACTACTTTG**
CDR 3

ACTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA

Protein sequence: (one-letter code)

EIQLQQSGPELVKPGASVKVSCKASGYSF**TDYSMY**WVKQSHGKSLEWIG**YIDPYNGGTRYNQ**
CDR 1 CDR 2

KFKGKATLTVDKSSSTAFMHLNSLTSEDSAVYYCAR**QTDYFDY**WGQGTTLTVSS
CDR 3

Light chain (variable domaine):

Nucleotide sequence:

GACATCCAGATGACACAGTCTCCATCCTCACTGTCTGCATCTCTGGGAGGCAAAGTCACCAT

CACTTGC**AAGGCAAGCCAAGACATTAACAATTATATAGCT**TGGTACCAACACAAGCCTGGAA
CDR 1

AAGGTCCTAGGCTGCTCATACAT**TACACATCTACATTACAAGCA**GGCATCCCATCAAGGTTC
CDR 2

AGTGGAAGTGGGTCTGGGAGAGATTATTCCTTCAGCATCAGCAACCTGGAGCCTGAAGATAT

TGGAACTTATTATTGT**CTACAGTATGATAATCTGTGGACG**TTCGGTGGAGGCACCAAGCTGG
CDR 3

AAATCAAA

Protein sequence: **(one-letter code)**

DIQMTQSPSSLSASLGGKVTITC<u>KASQDINNYIA</u>WYQHKPGKGPRLLIH<u>YTSTLQA</u>GIPSRF
CDR 1 CDR 2

SGSGSGRDYSFSISNLEPEDIGTYYC**LQYDNLWT**FGGGTKLEIK
CDR 3

FIGURE 1

IGKV19-93*01 (IMGT nomenclature): **98.56% (275/279 nt)**

```
                          <---------------------------------- FR1-IMGT ---------------
6F4 VL domain (AA)         D  I  Q  M  T  Q  S  P  S  S  L  S  A  S  L  G  G  K  V  T
6F4 VL domain             gacatccagatgacacagtctccatcctcactgtctgcatctctgggaggcaaagtcacc
AJ235935 IGKV19-93*01     ------------------------------------------------------------

                          -----------------> CDR1-IMGT <-----
6F4 VL domain (AA)         I  T  C  K  A  S  Q  D  I  N  N  Y                 I  A
6F4 VL domain             atcacttgcaaggcaagccaagacattaacaattat.................atagct
AJ235935 IGKV19-93*01     ---------------------------------g---.................------

                          --------------- FR2-IMGT ------------------>__________ CDR
6F4 VL domain (AA)         W  Y  Q  H  K  P  G  K  G  P  R  L  L  I  H  Y  T  S
6F4 VL domain             tggtaccaacacaagcctggaaaaggtcctaggctgctcatacattacacatct......
AJ235935 IGKV19-93*01     ------------------------------------------------------......

                          2-IMGT ________<-----------------------------------------------
6F4 VL domain (AA)                        T  L  Q  A  G  I  P     S  R  F  S  G  S  G
6F4 VL domain             ..............acattacaagcaggcatccca...tcaaggttcagtggaagtggg
AJ235935 IGKV19-93*01     ..............--------gc-----------...----------------------

                          --------- FR3-IMGT ---------------------------------------
6F4 VL domain (AA)               S  G  R  D  Y  S  F  S  I  S  N  L  E  P  E  D  I  G
6F4 VL domain             ......tctgggagagattattccttcagcatcagcaacctggagcctgaagatattgga
AJ235935 IGKV19-93*01     ......-------------------------------------------------c-

                          -----------> CDR3-IMGT _____<-------------- FR4-IMGT
6F4 VL domain (AA)         T  Y  Y  C  L  Q  Y  D  N  L  W  T  F  G  G  G  T  K  L  E
6F4 VL domain             acttattattgtctacagtatgataatctgtggacgttcggtggaggcaccaagctggaa
AJ235935 IGKV19-93*01     ------------------------------tctac-

                          ------>
6F4 VL domain (AA)         I  K
6F4 VL domain             atcaaac
```

FIGURE 2A

IGKJ1*01 (IMGT nomenclature): **100.0% (38/38 nt)**

```
                     CDR3-IMGT _____<----------- FR4-IMGT ------->
6F4 VL domain (AA)              W  T  F  G  G  G  T  K  L  E  I  K
6F4 VL domain                  gtggacgttcggtggaggcaccaagctggaaatcaaac
V00777 IGKJ1*01                -------------------------------------
```

FIGURE 2B

IGKV1-33*01 (IMGT nomenclature): **81.36% (227/279 nt)**

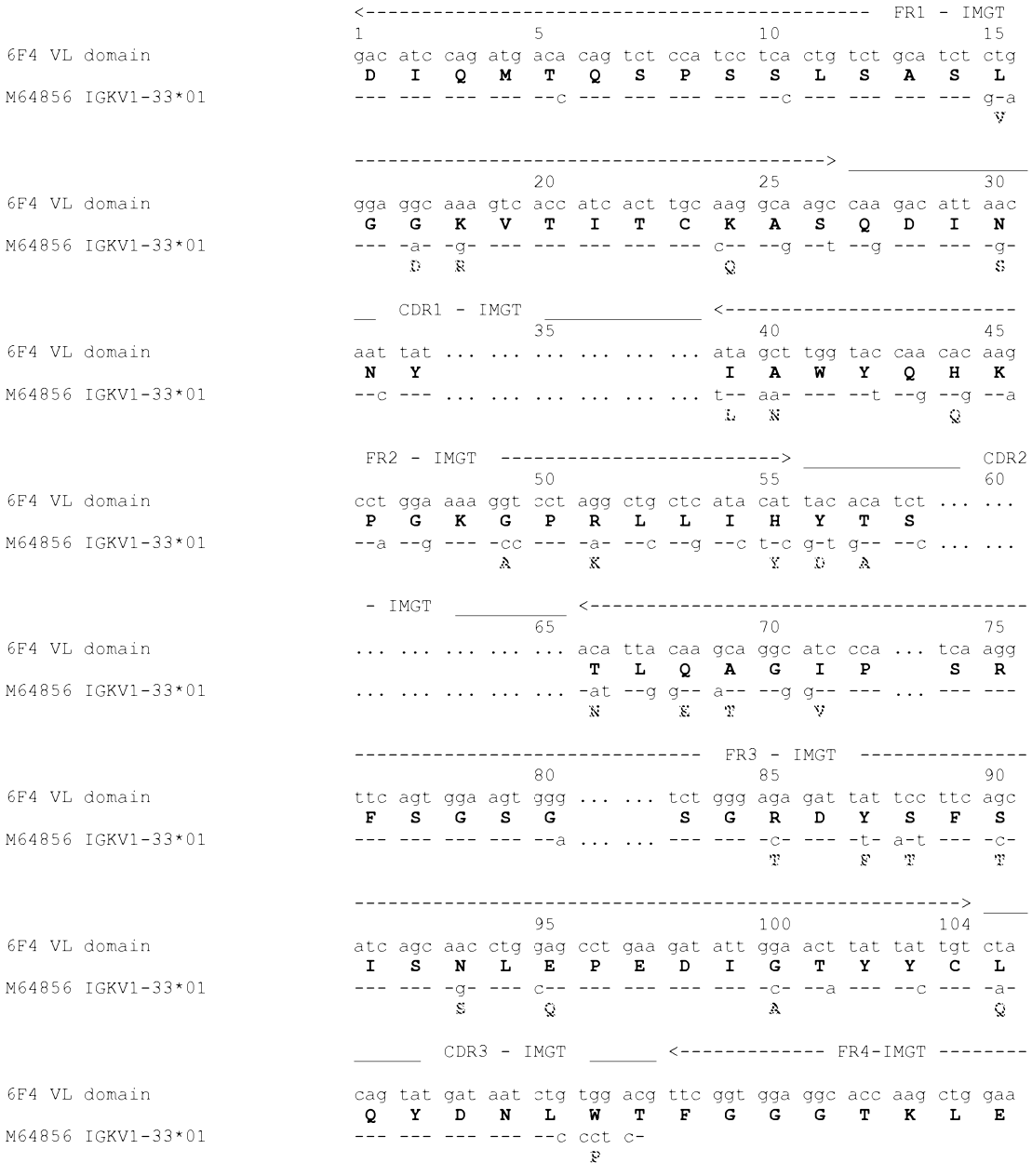


FIGURE 3A

IGKJ1*01 (IMGT nomenclature): **86.84% (33/38 nt)**

```
                                CDR3 - IMGT__ <-------------- FR4-IMGT ------------->
6F4 VL DOMAIN                       G TGG ACG TTC GGT GGA GGC ACC AAG CTG GAA ATC AAA
                                      W   T   F   G   G   G   T   K   L   E   I   K
J00242 IGKJ1*01                     - --- --- --- --C CA- --G --- --- G-- --- --- ---
                                                    Q               V
```

FIGURE 3B

Sequential numbering	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
IMGT unique numbering (1)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Kabat VL numbering (2)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
murine 6F4 VL	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	L	G	G	K	V	T	I	T

										CDR1												
Sequential numbering	23	24	25	26	27		28	29	30	31	32							33	34	35	36	37
IMGT unique numbering (1)	23	24	25	26	27		28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
Kabat VH numbering (2)	23	24	25	26	27	(A-F)	28	29	30	31	32							33	34	35	36	37
murine 6F4 VL	C	K	A	S	Q	-	D	I	N	N	Y	-	-	-	-	-	-	I	A	W	Y	Q

																CDR2						
Sequential numbering	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52							
IMGT unique numbering (1)	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65
Kabat VH numbering (2)	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52							
murine 6F4 VL	H	K	P	G	K	G	P	R	L	L	I	H	Y	T	S	-	-	-	-	-	-	-

Sequential numbering	53	54	55	56	57	58	59		60	61	62	63	64	65	66			67	68	69	70	71
IMGT unique numbering (1)	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87
Kabat VH numbering (2)	53	54	55	56	57	58	59		60	61	62	63	64	65	66			67	68	69	70	71
murine 6F4 VL	T	L	Q	A	G	I	P	-	S	R	F	S	G	S	G	-	-	S	G	R	D	Y

Sequential numbering	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	
IMGT unique numbering (1)	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109
Kabat VH numbering (2)	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	
murine 6F4 VL	S	F	S	I	S	N	L	E	P	E	D	I	G	T	Y	Y	C	L	Q	Y	D	-

	CDR3																					
Sequential numbering					93	94	95	96	97	98	99	100	101	102	103	104	105	106				
IMGT unique numbering (1)	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128			
Kabat VH numbering (2)					93	94	95	96	97	98	99	100	101	102	103	104	105	106				
murine 6F4 VL	-	-	-	-	N	L	W	T	F	G	G	G	T	K	L	E	I	K	-			

FIGURE 4

```
                                <---------------------------------- FR1-IMGT ---------------
6F4 VH domain (AA)               E  I  Q  L  Q  Q  S  G  P     E  L  V  K  P  G  A  S  V  K
6F4 VH domain                   gagatccagctgcagcagtctggacct...gagctggtgaagcctggggcttcagtgaag
AF304556 IGHV1S135*01           ----------------------------...------------------------------

                                -----------------> CDR1-IMGT <-----
6F4 VH domain (AA)               V  S  C  K  A  S  G  Y  S  F  T  D  Y  S              M  Y
6F4 VH domain                   gtatcctgcaaggcttctggttactcattcactgactacagc.............atgtac
AF304556 IGHV1S135*01           ----------------------------------------a-............------

                                --------------- FR2-IMGT ------------------> CDR
6F4 VH domain (AA)               W  V  K  Q  S  H  G  K  S  L  E  W  I  G  Y  I  D  P  Y  N
6F4 VH domain                   tgggtgaagcagagccatggaaagagccttgagtggattggatatattgatccttacaat
AF304556 IGHV1S135*01           ------------------------------------------------------------

                                2-IMGT <-----------------------------------------------
6F4 VH domain (AA)               G  G  T        R  Y  N  Q  K  F  K     G  K  A  T  L  T  V
6F4 VH domain                   ggtggtact......aggtacaaccagaagttcaag...ggcaaggccacattgactgtt
AF304556 IGHV1S135*01           ---------......--c------------------...---------------------

                                --------- FR3-IMGT ----------------------------------------
6F4 VH domain (AA)               D  K  S  S  S  T  A  F  M  H  L  N  S  L  T  S  E  D  S  A
6F4 VH domain                   gacaagtcctccagcacagccttcatgcatctcaacagcctgacatctgaggactctgca
AF304556 IGHV1S135*01           ------------------------------------------------------------

                                -----------> CDR3-IMGT <-------
6F4 VH domain (AA)               V  Y  Y  C  A  R  Q  T  D  Y  F  D  Y  W  G  Q  G  T  T  L
6F4 VH domain                   gtctattactgtgcaagacagacggactactttgactactggggccaaggcaccactctc
AF304556 IGHV1S135*01           ------------------

6F4 VH domain (AA)               T  V  S  S
6F4 VH domain                   acagtctcctcag
```

FIGURE 5A

IGHD-ST4*01 (IMGT nomenclature): **80.00% (4/5 nt)**

```
                                    ____ CDR3-IMGT ___
6F4 VH domain (AA)                   A  R  Q  T  D  Y
6F4 VH domain                           cagacg
M23243 IGHD-ST4*01                      .----agctcgggctac
```

FIGURE 5B

IGHJ2*01 (IMGT nomenclature): **100.00% (48/48 nt)**

```
                                  ______ CDR3-IMGT ______<-------------FR4------------>
6F4 VL domain (AA)                 Q  T  D  Y  F  D  Y  W  G  Q  G  T  T  L  T  V  S  S
6F4 VH domain                     cagacggactactttgactactggggccaaggcaccactctcacagtctcctca
V00770 IGHJ2*01                   .......-----------------------------------------------
```

FIGURE 5C

IGHV1-f*01 (IMGT nomenclature): **75.34% (217/288 nt)**

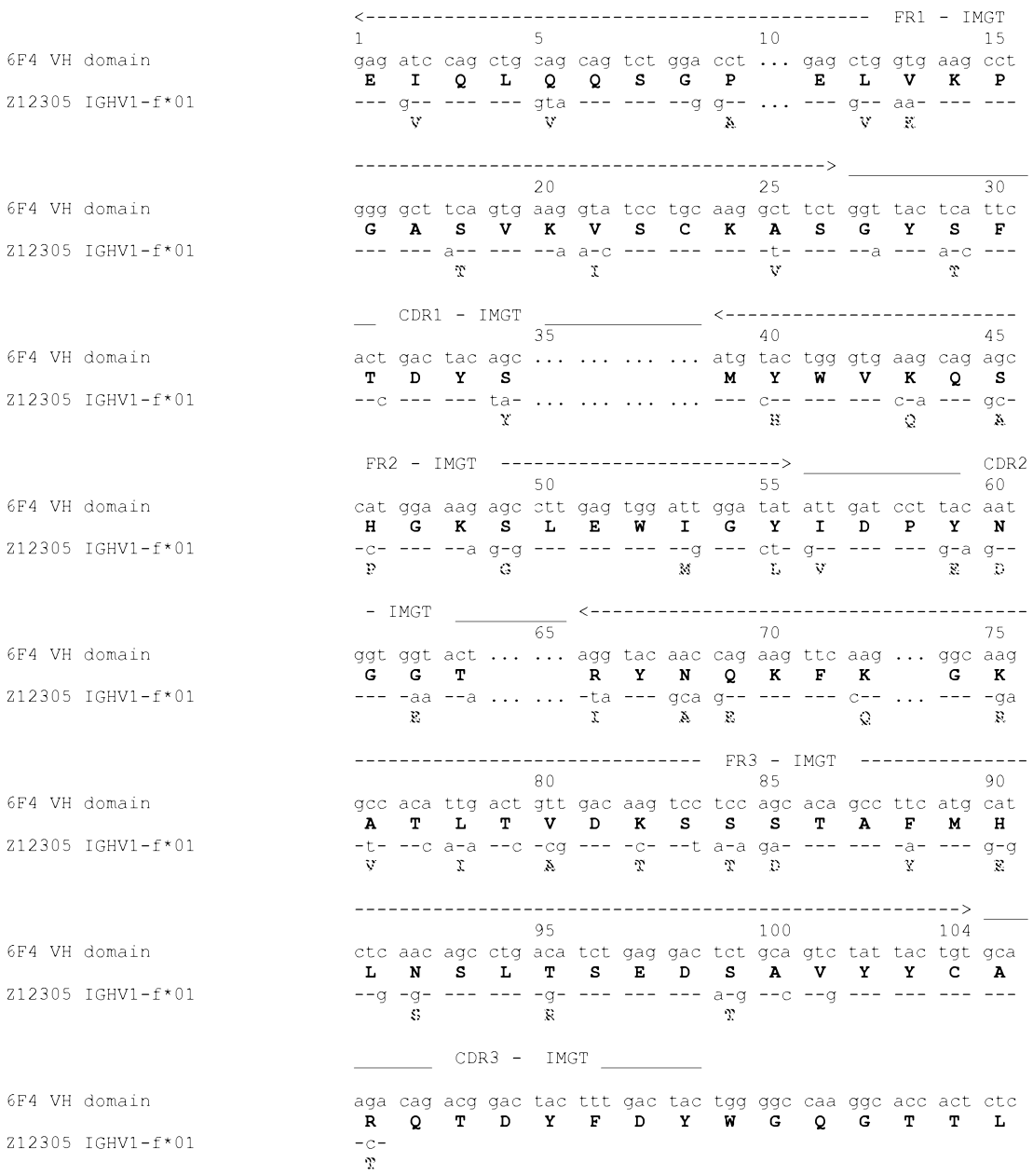


FIGURE 6A

IGHD1-1*01 (IMGT nomenclature): **71.42% (5/7 nt)**

```
                                  ___ CDR3 - IMGT ___
6F4 VH domain                     cag acg gac tac ttt
                                   Q   T   D   Y   F
X97051 IGHD1-1*01                  - -ac ---
                                     N
```

FIGURE 6B

IGHJ4*01 (IMGT nomenclature): **87.50% (42/48 nt)**

```
                    __________ CDR3-IMGT __________<------------------ FR4 -------------->
6F4 VH domain       gacagacg gac tac ttt gac tac tgg ggc caa ggc acc act ctc aca gtc tcc tca
                             D   Y   F   D   Y   W   G   Q   G   T   T   L   T   V   S   S
J00256 IGHJ4*01     ........ .-- --- --- --- --- --- --- --- --a --- ctg g-- --c --- --- ---
                                                                      L   V
```

FIGURE 6C

Sequential numbering	1	2	3	4	5	6	7	8	9		10	11	12	13	14	15	16	17	18	19	20	21
IMGT unique numbering (1)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Kabat VL numbering (2)	1	2	3	4	5	6	7	8	9		10	11	12	13	14	15	16	17	18	19	20	21
murine 6F4 VH	E	I	Q	L	Q	Q	S	G	P	-	E	L	V	K	P	G	A	S	V	K	V	S

											CDR1											
Sequential numbering	22	23	24	25	26	27	28	29	30	31	32	33					34	35		36	37	38
IMGT unique numbering (1)	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40		41	42	43
Kabat VL numbering (2)	22	23	24	25	26	27	28	29	30	31	32	33					34	35	(A-B)	36	37	38
murine 6F4 VH	C	K	A	S	G	Y	S	F	T	D	Y	S	-	-	-	-	M	Y	-	W	V	K

																			CDR2			
Sequential numbering	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53		54	55	56	57	58	
IMGT unique numbering (1)	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58		59	60	61	62	63	64
Kabat VH numbering (2)	39	40	41	42	43	44	45	46	47	48	49	50	51	52	52A	(B-C)	53	54	55	56	57	
murine 6F4 VH	Q	S	H	G	K	S	L	E	W	I	G	Y	I	D	P		Y	N	G	G	T	-

Sequential numbering		59	60	61	62	63	64	65		66	67	68	69	70	71	72	73	74	75	76	77	78
IMGT unique numbering (1)	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86
Kabat VH numbering (2)		58	59	60	61	62	63	64		65	66	67	68	69	70	71	72	73	74	75	76	77
murine 6F4 VH	-	R	Y	N	Q	K	F	K		G	K	A	T	L	T	V	D	K	S	S	S	T

Sequential numbering	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
IMGT unique numbering (1)	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108
Kabat VH numbering (2)	78	79	80	81	82	82A	82B	82C	83	84	85	86	87	88	89	90	91	92	93	94	95	96
murine 6F4 VH	A	F	M	H	L	N	S	L	T	S	E	D	S	A	V	Y	Y	C	A	R	Q	T

	CDR3																					
Sequential numbering	101					102	103	104	105	106	107	108	109	110	111	112	113	114	115	116		
IMGT unique numbering (1)	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128		
Kabat VH numbering (2)	97					98	99	100	101	102	103	104	105	106	107	108	109	110	111	112		
murine 6F4 VH	D	-	-	-	-	Y	F	D	Y	W	G	Q	G	T	T	L	T	V	S	S		

FIGURE 7

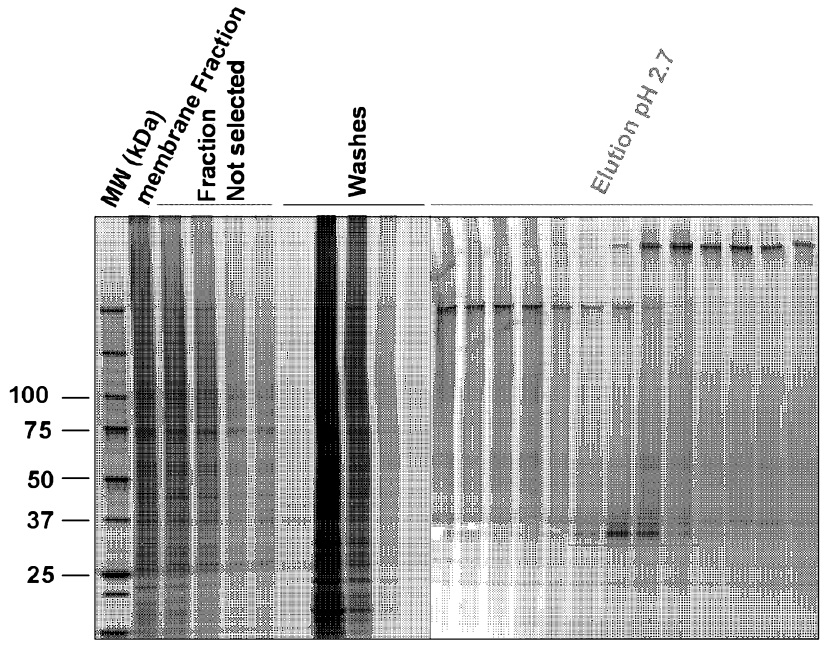


FIGURE 8A

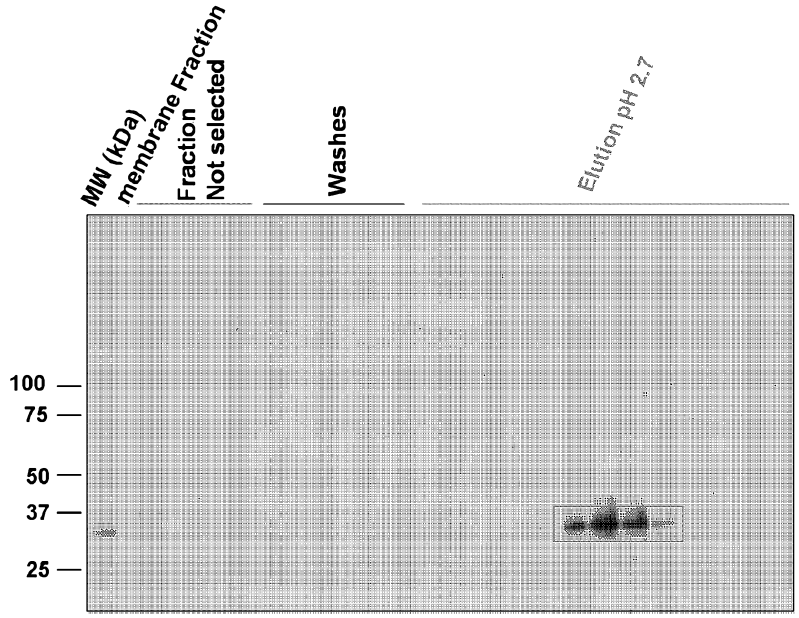


FIGURE 8B

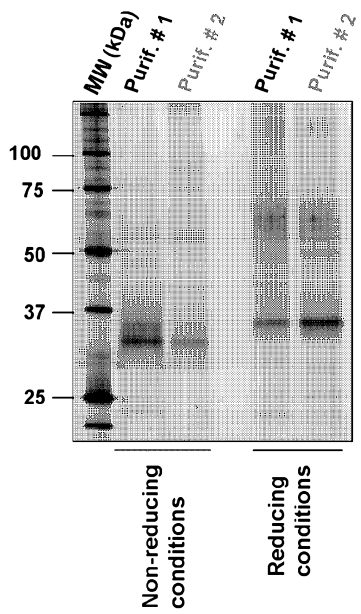


FIGURE 9A

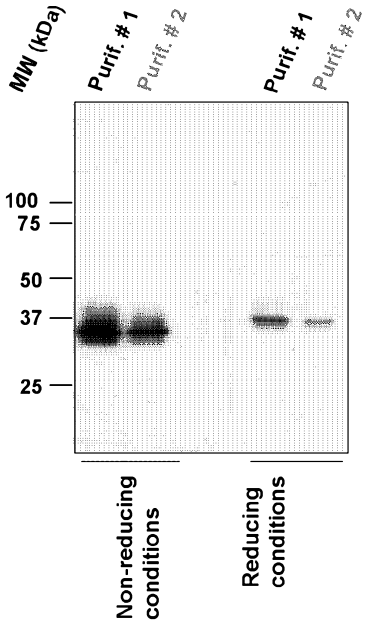


FIGURE 9B

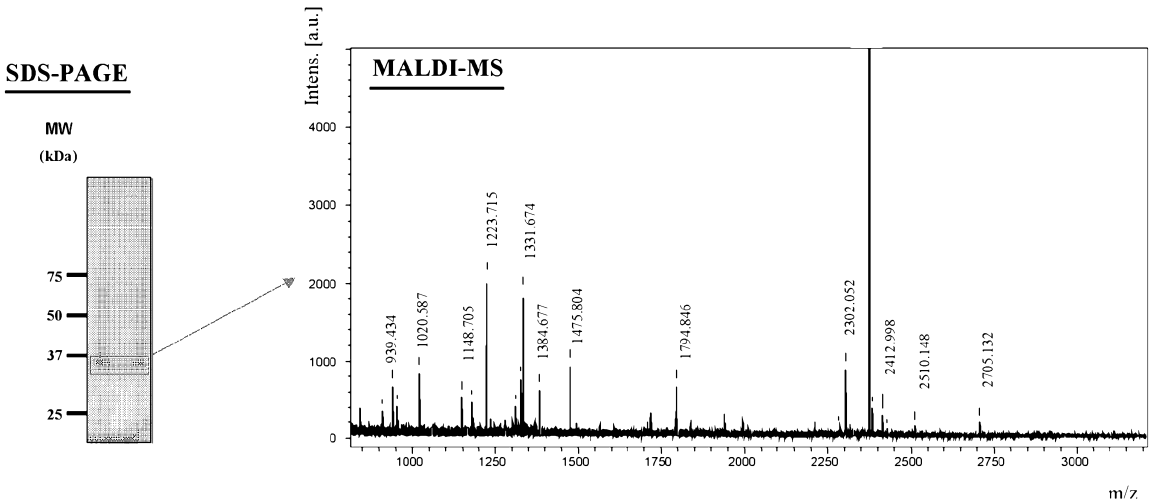


FIGURE 10

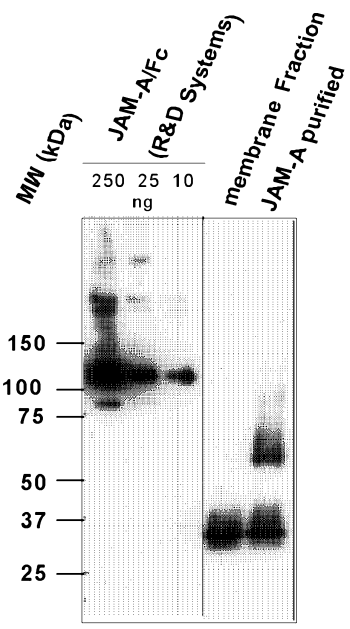


FIGURE 11A

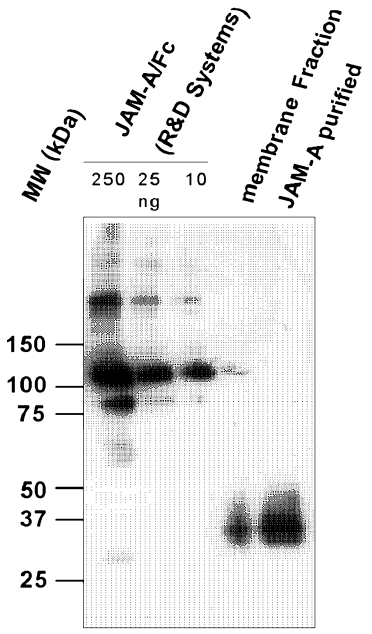


FIGURE 11B

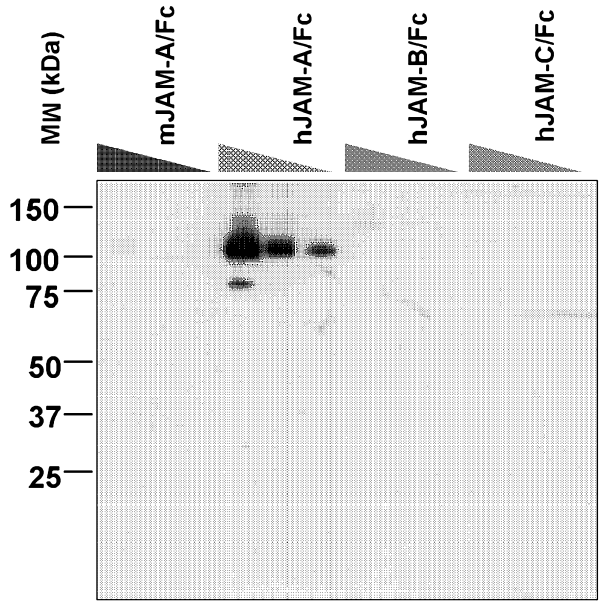


FIGURE 12

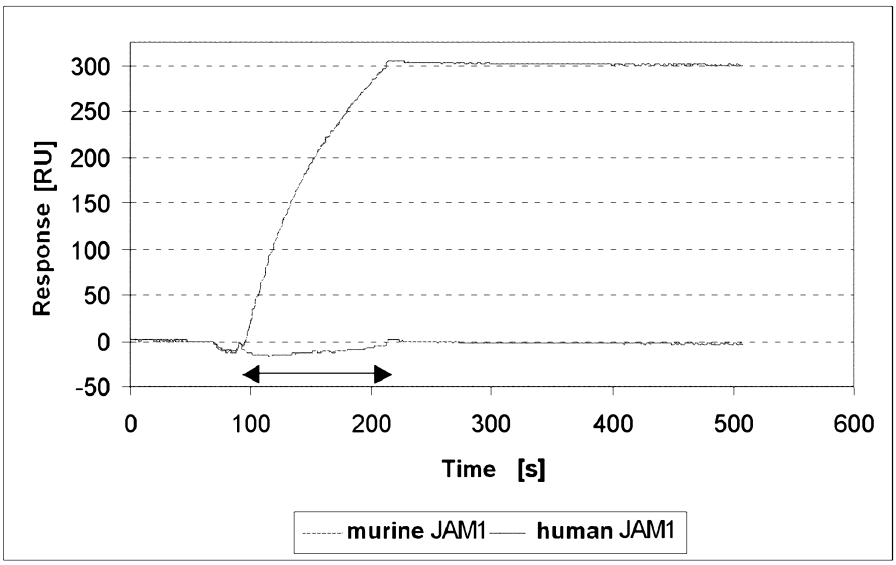


FIGURE 13

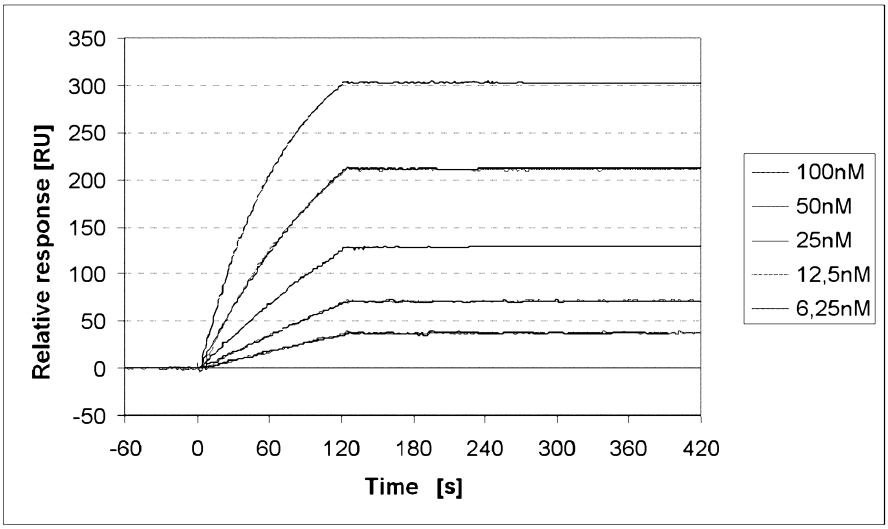


FIGURE 14

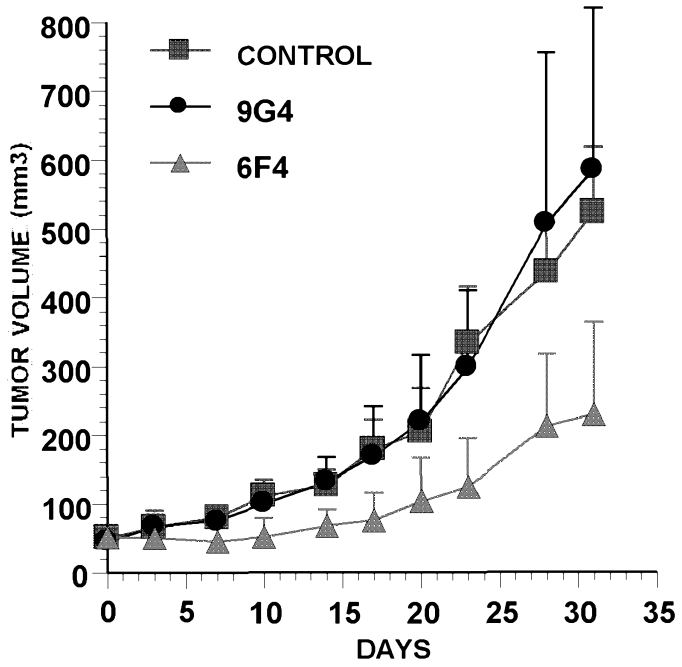


FIGURE 15

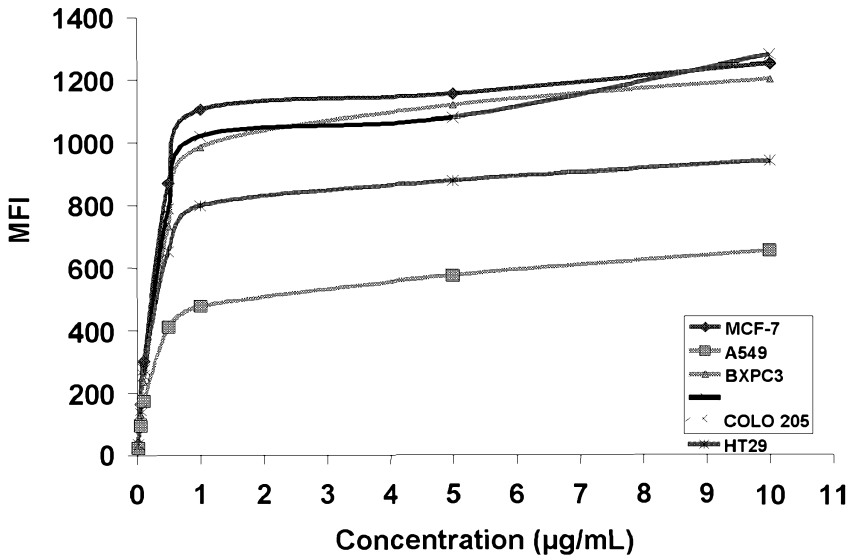
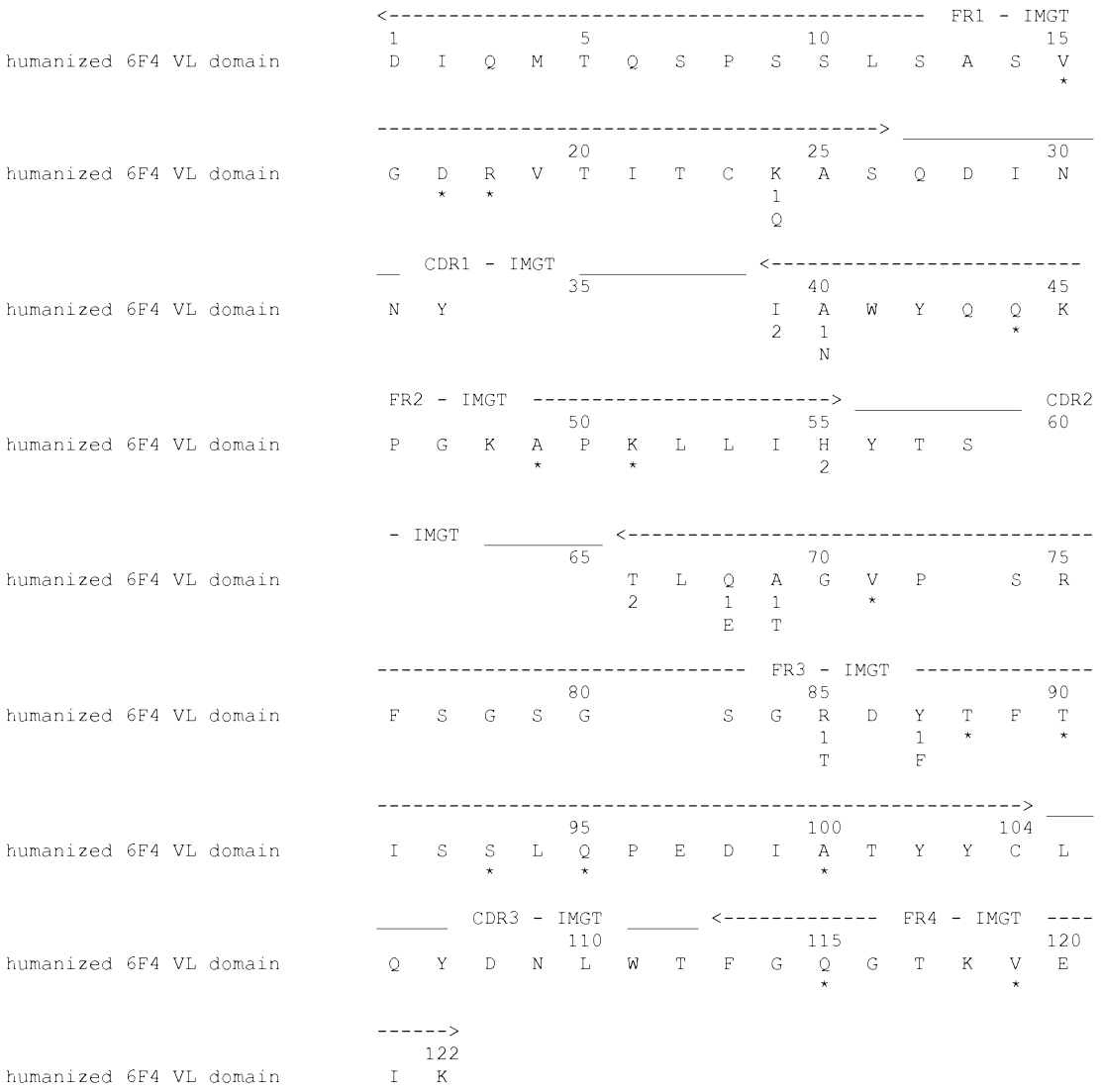


FIGURE 16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1 5 10 15
humanized 6F4 VL domain D I Q M T Q S P S S L S A S V
*
----------> _____
20 25 30
humanized 6F4 VL domain G D R V T I T C K A S Q D I N
* * 1
Q
__ CDR1 - IMGT _____ <----------
35 40 45
humanized 6F4 VL domain N Y I A W Y Q Q K
2 1 *
N
FR2 - IMGT ----------> _____ CDR2
50 55 60
humanized 6F4 VL domain P G K A P K L L I H Y T S
* * 2
- IMGT _____ <----------
65 70 75
humanized 6F4 VL domain T L Q A G V P S R
2 1 1 *
E T
---------- FR3 - IMGT ----------
80 85 90
humanized 6F4 VL domain F S G S G S G R D Y T F T
1 1 * *
T F
----------> ____
95 100 104
humanized 6F4 VL domain I S S L Q P E D I A T Y Y C L
* * *
_____ CDR3 - IMGT _____ <---------- FR4 - IMGT ----
110 115 120
humanized 6F4 VL domain Q Y D N L W T F G Q G T K V E
* *
------>
122
humanized 6F4 VL domain I K

FIGURE 17

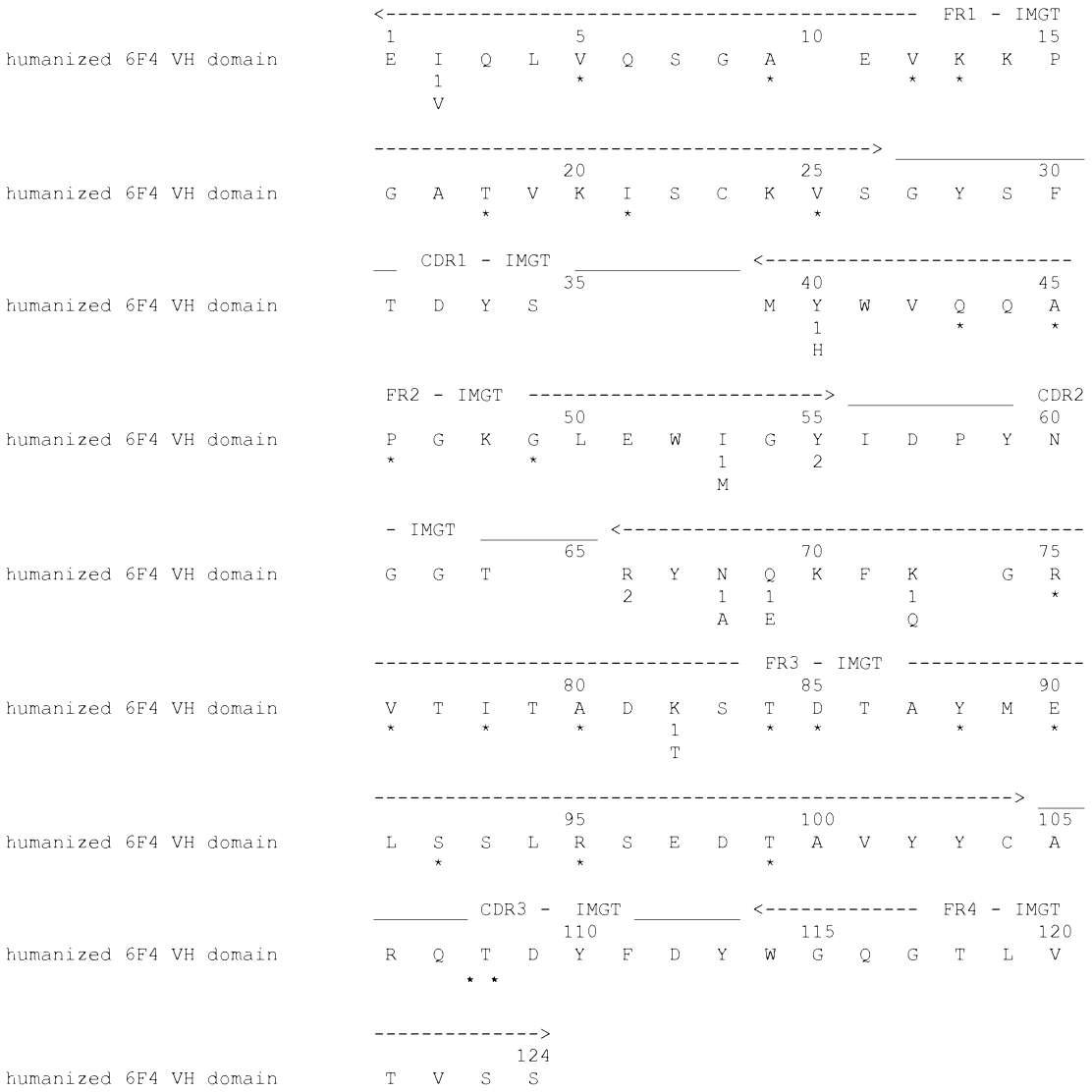

<---------- FR1 - IMGT
1 5 10 15
humanized 6F4 VH domain E I Q L V Q S G A E V K K P
1 * * * *
V
---------->
20 25 30
humanized 6F4 VH domain G A T V K I S C K V S G Y S F
* * *
__ CDR1 - IMGT <----------
35 40 45
humanized 6F4 VH domain T D Y S M Y W V Q Q A
1 * *
H
FR2 - IMGT ----------> CDR2
50 55 60
humanized 6F4 VH domain P G K G L E W I G Y I D P Y N
* * 1 2
M
- IMGT <----------
65 70 75
humanized 6F4 VH domain G G T R Y N Q K F K G R
2 1 1 1 *
A E Q
---------- FR3 - IMGT ----------
80 85 90
humanized 6F4 VH domain V T I T A D K S T D T A Y M E
* * * 1 * * * *
T
---------->
95 100 105
humanized 6F4 VH domain L S S L R S E D T A V Y Y C A
* * *
CDR3 - IMGT <---------- FR4 - IMGT
110 115 120
humanized 6F4 VH domain R Q T D Y F D Y W G Q G T L V
* *
---------->
124
humanized 6F4 VH domain T V S S

FIGURE 18

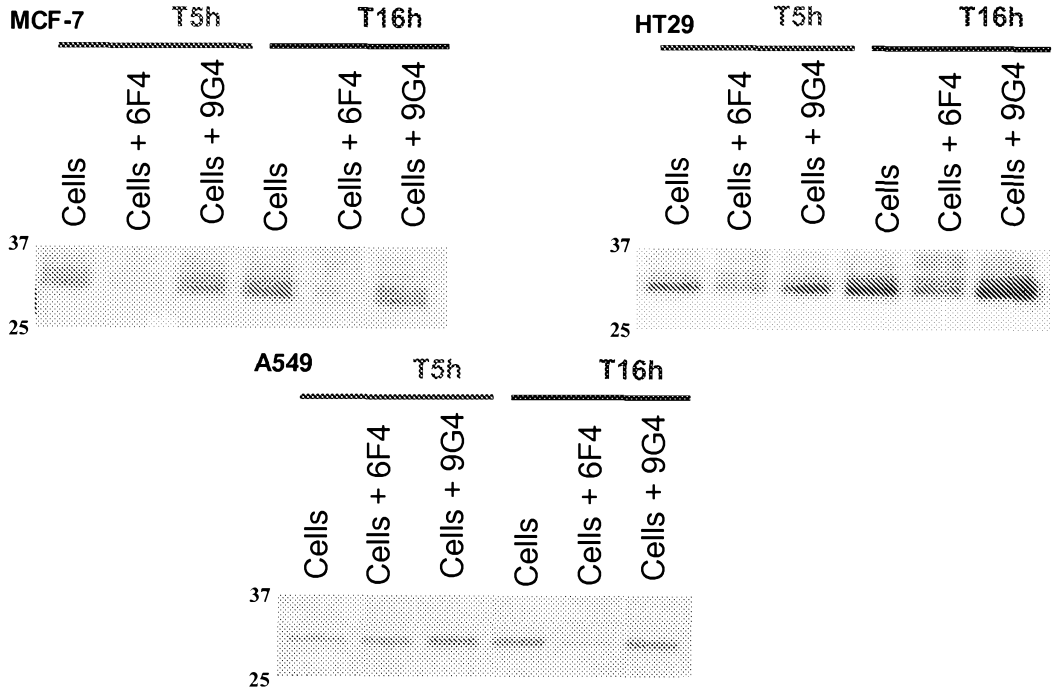


FIGURE 19

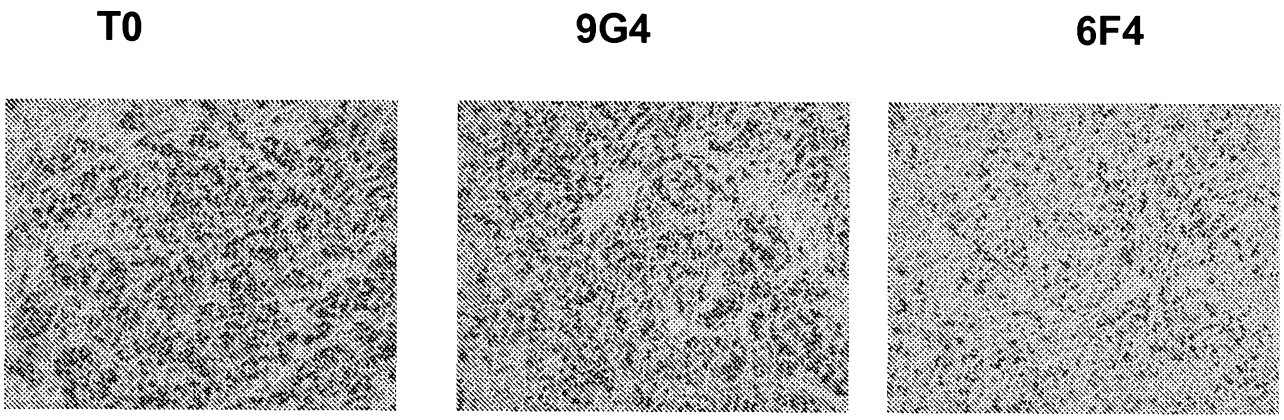


FIGURE 20

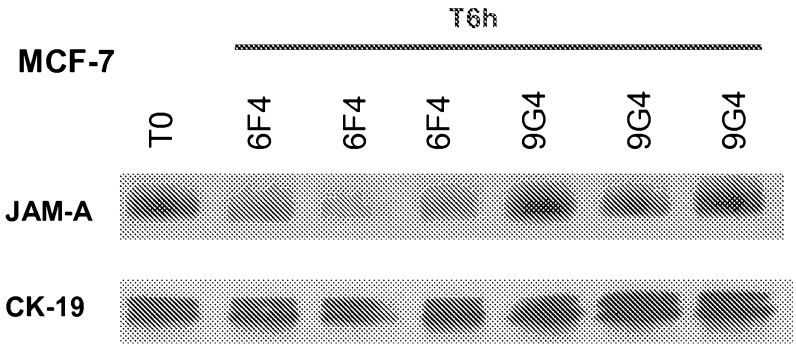


FIGURE 21

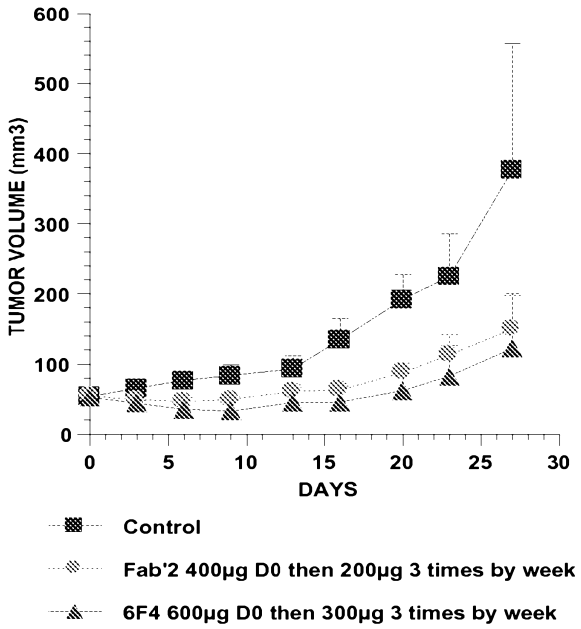


FIGURE 22

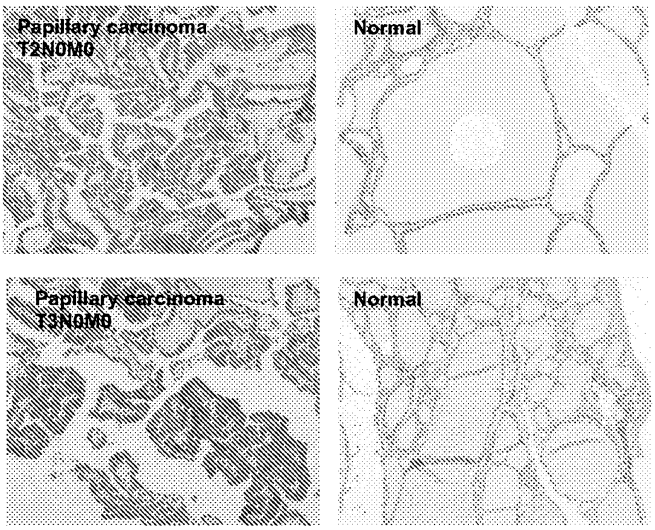


FIGURE 23

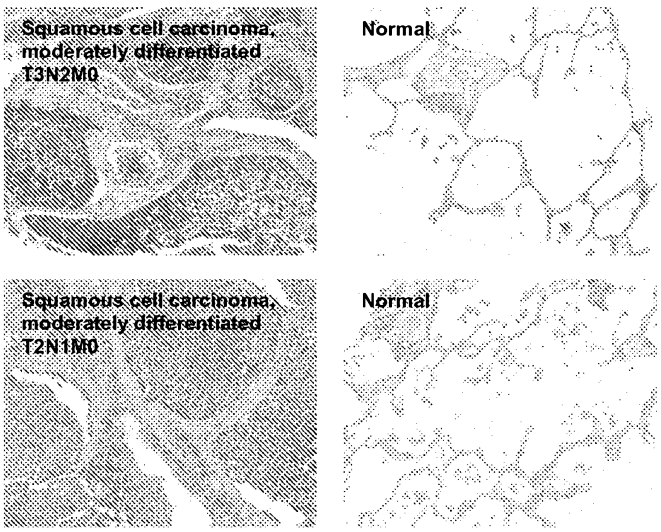


FIGURE 24

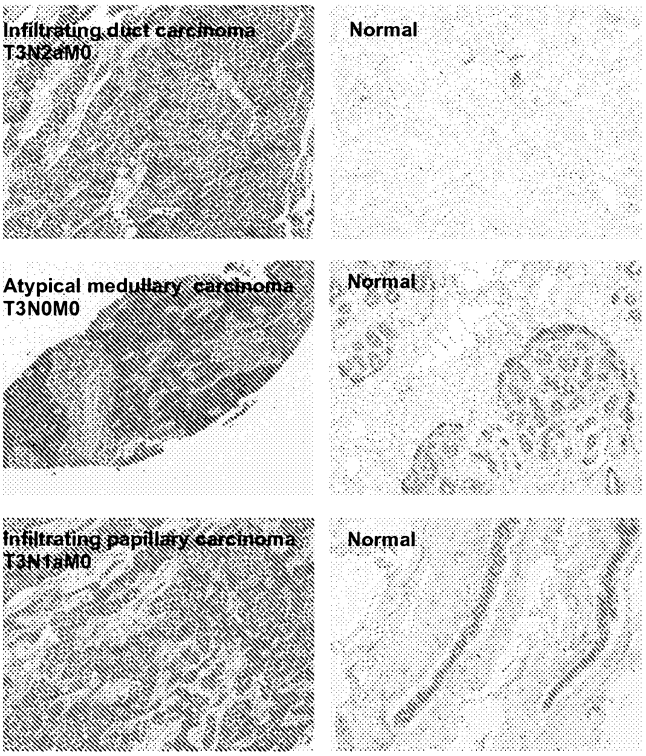


FIGURE 25

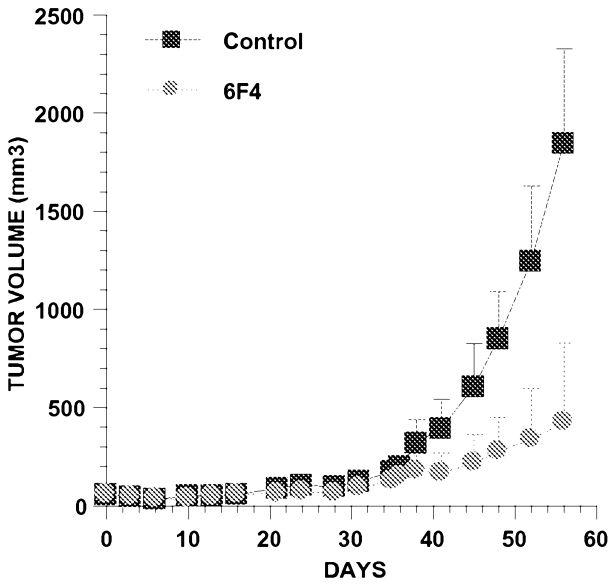
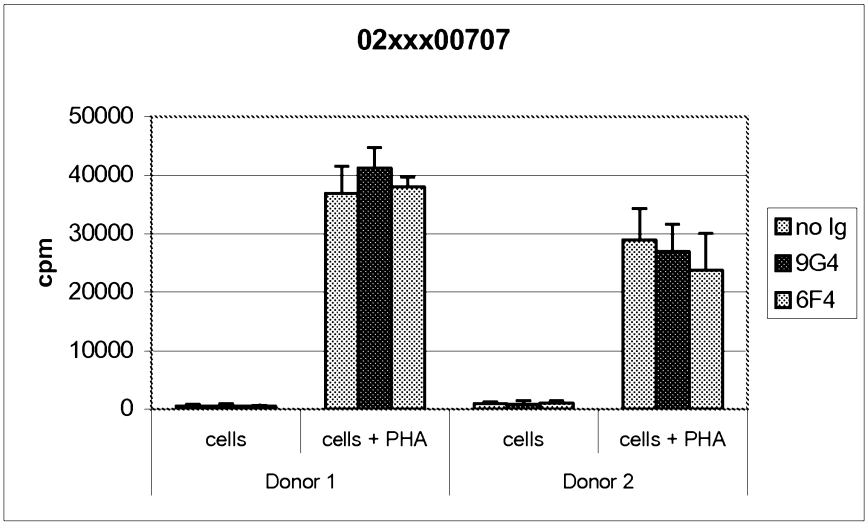
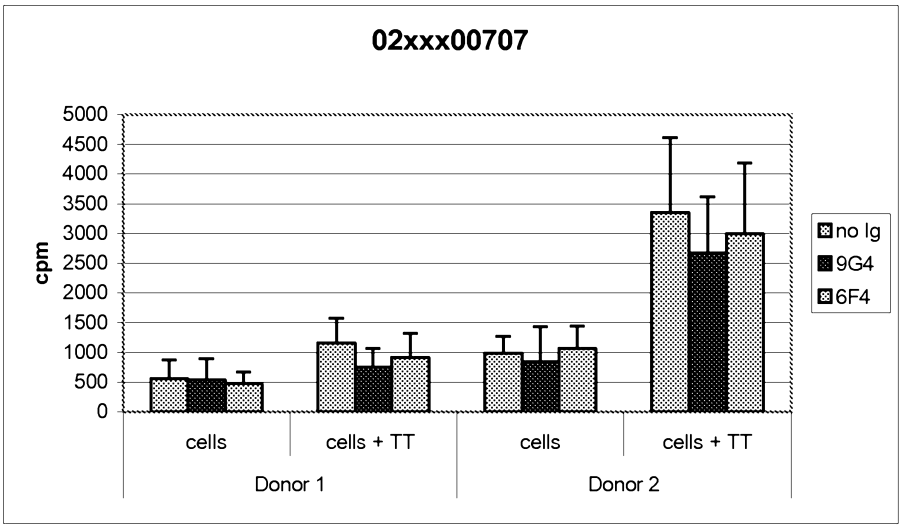


FIGURE 26

A


02xxx00707
50000
40000
30000
20000
10000
0
cpm
cells
cells + PHA
cells
cells + PHA
Donor 1
Donor 2
no Ig
9G4
6F4

B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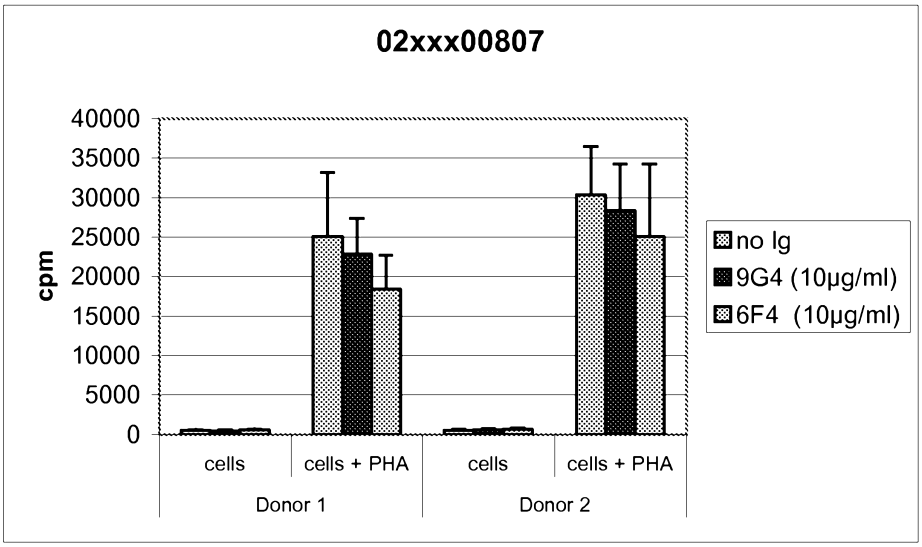
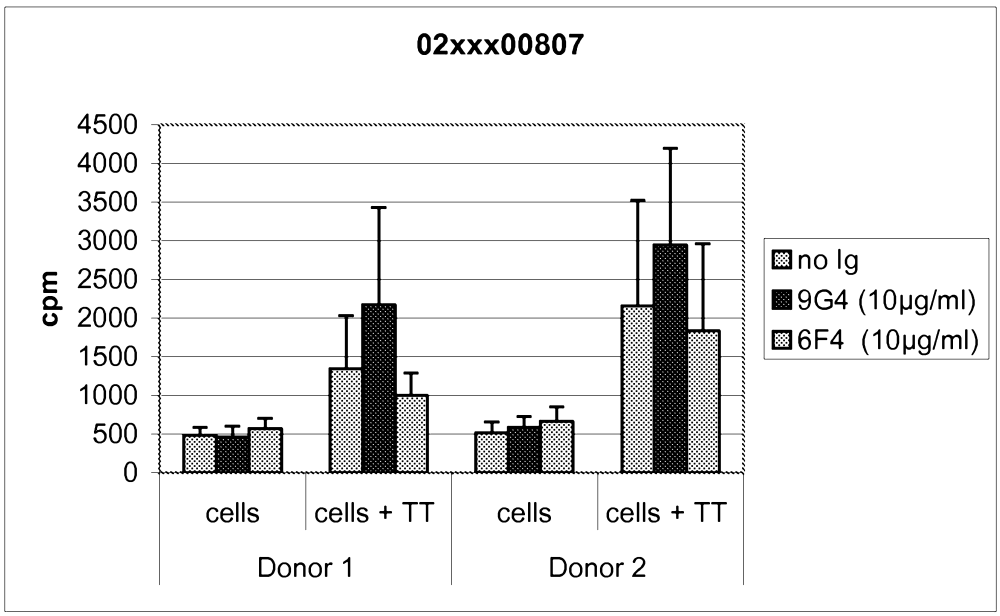
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
cpm
cells
cells + TT
cells
cells + TT
Donor 1
Donor 2
no Ig
9G4
6F4

FIGURE 27

A


02xxx00807
cpm
40000
35000
30000
25000
20000
15000
10000
5000
0
cells
cells + PHA
cells
cells + PHA
Donor 1
Donor 2
no Ig
9G4 (10µg/ml)
6F4 (10µg/ml)

B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cpm
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4000
3500
3000
2500
2000
1500
1000
500
0
cells
cells + TT
cells
cells + TT
Donor 1
Donor 2
no Ig
9G4 (10µg/ml)
6F4 (10µg/ml)

FIGURE 28

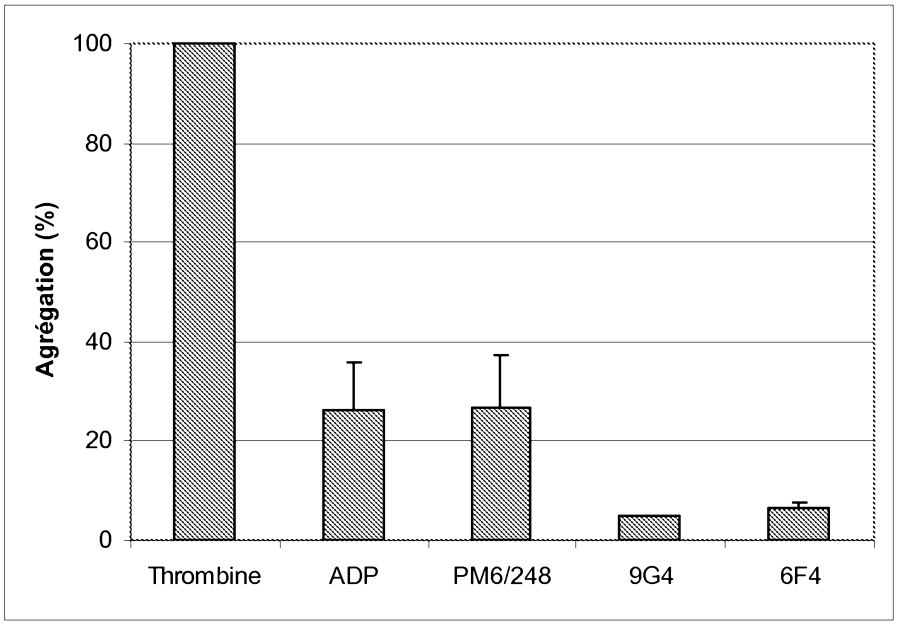


FIGURE 29

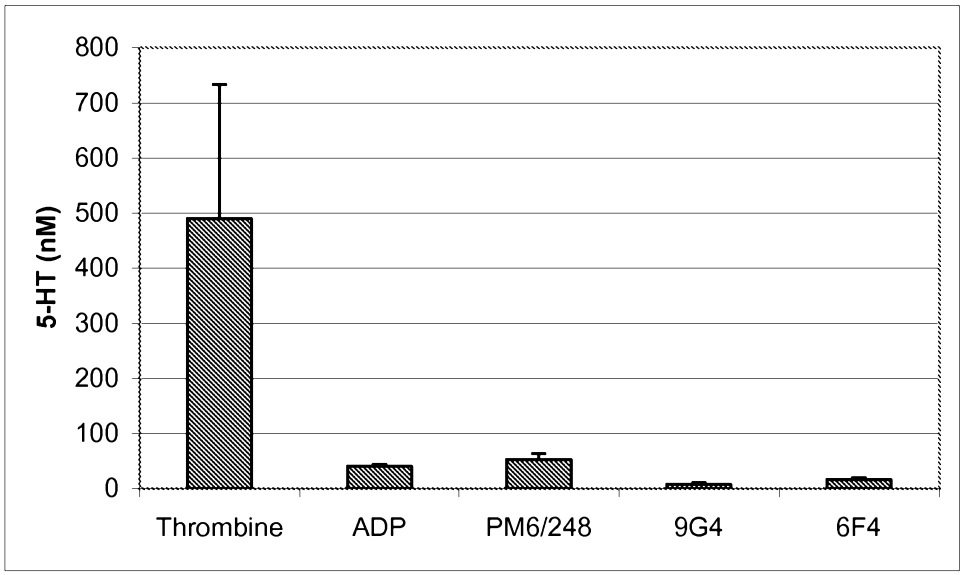
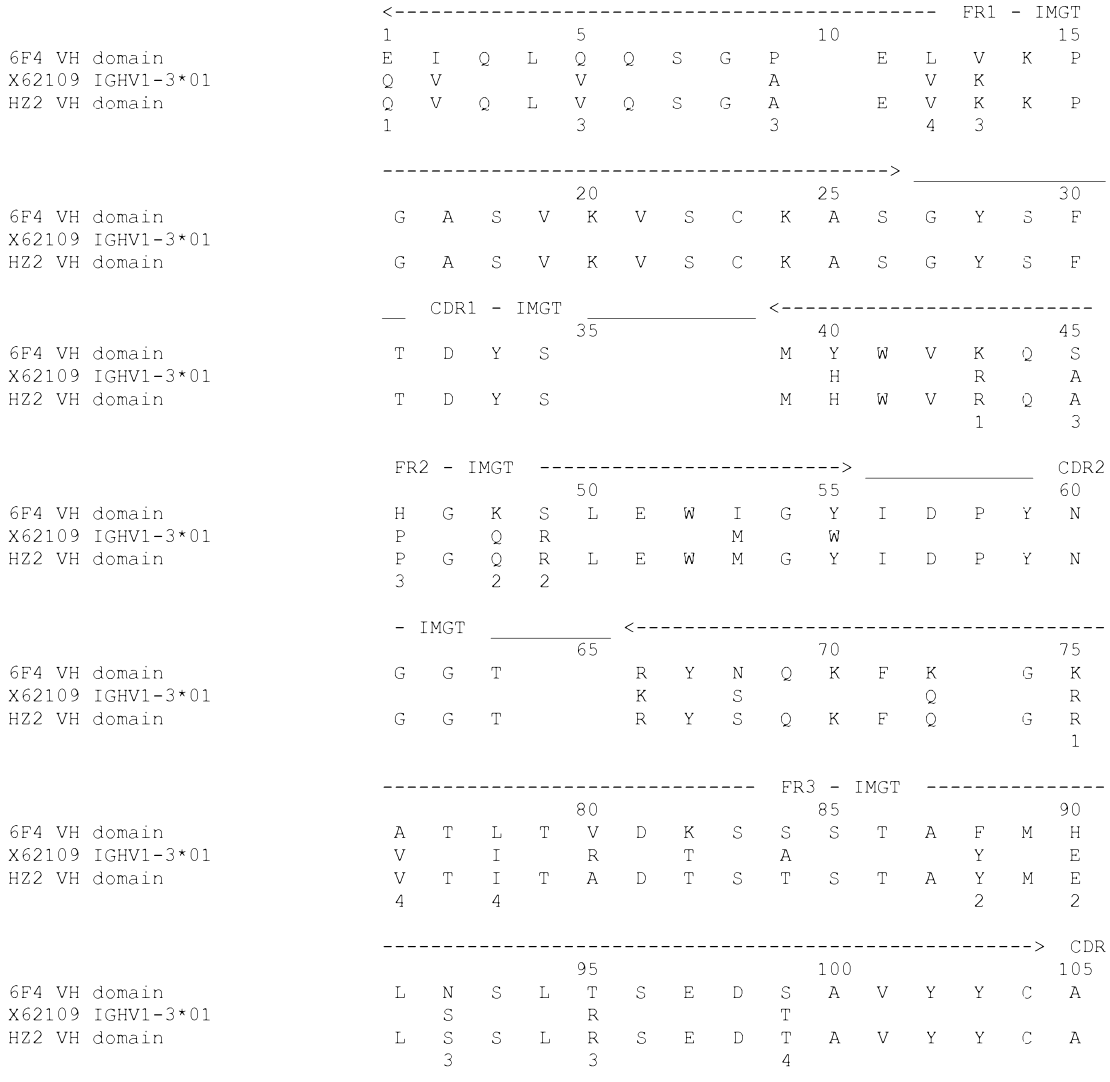


FIGURE 30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CDR1 - IMGT
FR2 - IMGT
CDR2
- IMGT
FR3 - IMGT
CDR
6F4 VH domain
X62109 IGHV1-3*01
HZ2 VH domain

FIGURE 31

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

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<170> PatentIn version 3.3

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1 5 10 15
Gly

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<212> PRT
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1 5

<210> 13
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Gly Lys Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Asn Asn Tyr
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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

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Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Asp Tyr
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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 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
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<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

<400> 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 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
```

<210> 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

attgatcctt 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 tcactgacta 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

tatattgatc cttacaatgg tggtactagg 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
ggaaaaggtc ctaggctgct catacattac acatctacat tacaagcagg catcccatca 180
aggttcagtg gaagtgggtc tgggagagat tattccttca gcatcagcaa cctggagcct 240
gaagatattg gaacttatta ttgtctacag tatgataatc tgtggacgtt cggtggaggc 300
accaagctgg aaatcaaa 318

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

<400> 33

gagatccagc tgcagcagtc tggacctgag ctggtgaagc ctggggcttc agtgaaggta 60
tcctgcaagg cttctggtta ctcattcact gactacagca tgtactgggt gaagcagagc 120
catggaaaga gccttgagtg gattggatat attgatcctt acaatggtgg tactaggtac 180
aaccagaagt tcaagggcaa ggccacattg actgttgaca agtcctccag cacagccttc 240
atgcatctca acagcctgac atctgaggac tctgcagtct attactgtgc aagacagacg 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
ggaaaaggtc ctaggctgct catacattac acatctacat tacaagcagg catcccatca 180
aggttcagtg gaagtgggtc tgggagagat tattccttca gcatcagcaa cctggagcct 240
gaagatattg gaacttatta ttgtctacag tatgataatc tgtggacgtt cggtggaggc 300
accaagctgg aaatcaaacg ggctgatgct gcaccaactg tatccatctt cccaccatcc 360
agtgagcagt taacatctgg aggtgcctca gtcgtgtgct tcttgaacaa cttctacccc 420
aaagacatca atgtcaagtg gaagattgat ggcagtgaac gacaaaatgg cgtcctgaac 480
agttggactg atcaggacag caaagacagc acctacagca tgagcagcac cctcacgttg 540
accaaggacg agtatgaacg acataacagc tatacctgtg aggccactca caagacatca 600
acttcaccca ttgtcaagag cttcaacagg aatgagtgt 639

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

<213> mus musculus

<400> 35

gagatccagc tgcagcagtc tggacctgag ctggtgaagc ctggggcttc agtgaaggta 60
tcctgcaagg cttctggtta ctcattcact gactacagca tgtactgggt gaagcagagc 120
catggaaaga gccttgagtg gattggatat attgatcctt acaatggtgg 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 ggcccctgga tctgctgccc aaactaactc catggtgacc 420
ctgggatgcc tggtcaaggg ctatttccct gagccagtga cagtgacctg gaactctgga 480
tccctgtcca gcggtgtgca caccttccca gctgtcctgc agtctgacct ctacactctg 540
agcagctcag tgactgtccc ctccagcacc tggcccagcg agaccgtcac ctgcaacgtt 600
gcccacccgg ccagcagcac caaggtggac aagaaaattg tgcccaggga ttgtggttgt 660
aagccttgca tatgtacagt cccagaagta tcatctgtct tcatcttccc cccaaagccc 720
aaggatgtgc tcaccattac tctgactcct aaggtcacgt gtgttgtggt agacatcagc 780
aaggatgatc ccgaggtcca gttcagctgg tttgtagatg atgtggaggt gcacacagct 840
cagacgcaac cccgggagga gcagttcaac agcactttcc gctcagtcag tgaacttccc 900
atcatgcacc aggactggct caatggcaag gagttcaaat gcagggtcaa cagtgcagct 960
ttccctgccc ccatcgagaa aaccatctcc aaaaccaaag gcagaccgaa ggctccacag 1020
gtgtacacca ttccacctcc caaggagcag atggccaagg ataaagtcag tctgacctgc 1080
atgataacag acttcttccc tgaagacatt actgtggagt ggcagtggaa tgggcagcca 1140
gcggagaact acaagaacac tcagcccatc atggacacag atggctctta cttcgtctac 1200
agcaagctca atgtgcagaa gagcaactgg gaggcaggaa atactttcac ctgctctgtg 1260
ttacatgagg gcctgcacaa ccaccatact gagaagagcc tctcccactc tcctggtaaa 1320

<210> 36
<211> 318
<212> DNA
<213> mus musculus

<400> 36

gacatacaga tgactcagag cccatcatca ttgagcgcgt ctgtcggcga tcgggttacc 60
attacctgcc aggcaagtca agatatcaac aactatattg cttggtatca acagaagccc 120
ggtaaagccc caaagctgct gatacactac acctccaccc tggagaccgg cgtgccttct 180
agattttctg gaagcgggtc cggaaccgat tatacgttca caatctccag ccttcagccc 240
gaagacatcg ccacatacta ctgtctgcaa tacgacaatc tgtggacatt tggccagggg 300
actaaggtgg agatcaaa 318

<210> 37
<211> 345
<212> DNA
<213> mus musculus

<400> 37

gaagtgcagc tggttcagag cggcgccgag gtaaaaccag gggcgacggt gaagataagc 60
tgcaaggtga gtgggtactc attcaccgac tattcaatgc actgggtcca acaggccccc 120
ggtaaaggac tggagtggat gggatacatc gatccctaca atggaggcac taggtacgcc 180
gagaagttcc aggggagagt cactattacc gcagatactt ctaccgatac tgcctacatg 240
gaactcagca gtctgcggtc cgaggacaca gcagtctact attgtgctcg ccaaacagac 300
tattttgact attggggcca gggaaccttg gtgacagtgt cctct 345

<210> 38
<211> 348
<212> DNA

<213> mus musculus

<400> 38

caggtgcaat tggtacagtc aggcgcggag gtgaagaagc ctggggctag tgttaaagtc 60
tcctgtaaag cctccggata ttccttcact gactactcta tgcattgggt tcgccaggca 120
ccagggcagc ggctggaatg gatggggtac attgatccct acaacggagg cacgcgatat 180
agtcagaagt tccagggtcg ggtgacaatc acagccgata cgtccaccag caccgcctac 240
atggagttga gcagtctcag gtcagaagac acagccgtgt actattgcgc aagacagacc 300
gattatttcg actactgggg ccaaggcact ctcgtgaccg 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 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

<400> 45

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

<210> 46
<211> 98
<212> PRT
<213> homo sapiens

<400> 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

<210> 47
<211> 121
<212> PRT
<213> homo sapiens

<400> 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

<210> 48
<211> 15
<212> PRT
<213> homo sapiens

<400> 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 tgatttgcat gtgctgagat catattctac tgccccagag atttaataat     60
ctgatcatac acactccaac agtcattctt ggtcaggaga cgttgtagaa atgagaccgt    120
ctattcagtt cctggggctc ttgttgttct ggcttcatgg taaggagttt aacattgaat    180
atgctaaaaa gagtatgtga tcaggaattt ctggtccttc 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 tcatacatta cacatctaca ttacagccag gcatcccatc aaggttcagt    480
ggaagtgggt ctgggagaga ttattccttc agcatcagca acctggagcc tgaagatatt    540
gcaacttatt attgtctaca gtatgataat cttctaccca cagtgataca aatcataaca    600
aaaaccaccc agggaagcag aagtgagagg 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
```

<400> 52

ctgcagctgt gcccagcctg ccctatcccc tgctgatttg catgttcgca gagcacagcc 60
ccctgccctg aagacttatt aataggctgg tcgcaccctg tgcaggagtc agtcccaacc 120
aggacacagc atggacatga gggtccctgc tcagctcctg gggctcctgc agctctggct 180
ctcaggtaag gaaggataac actaggaatt ttctcagcca gtgtgctcag tacagcctgg 240
ctcttgatgg aagccttcct ataatatgac taatagtatg aatatttgtg tttatgtttc 300
taatcgcagg tgccagatgt gacatccaga tgacccagtc tccatcctcc ctgtctgcat 360
ctgtaggaga cagagtcacc atcacttgcc aggcgagtca ggacattagc aactatttaa 420
attggtatca gcagaaacca gggaaagccc ctaagctcct gatctacgat gcatccaatt 480
tggaaacagg ggtcccatca aggttcagtg gaagtggatc tgggacagat tttactttca 540
ccatcagcag cctgcagcct gaagatattg caacatatta ctgtcaacag tatgataatc 600
tcccctcccac agtgtaacaa gtcataacat aaatcaccca ggggagcaga tgcgtgaggc 660
tcagctg 667

<210> 53
<211> 37
<212> DNA
<213> homo sapiens

<400> 53

tggacgttcg gccaagggac caaggtggaa atcaaac 37

<210> 54
<211> 294
<212> DNA
<213> mus musculus

<400> 54

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

<210> 55
<211> 163
<212> DNA
<213> mus musculus

<400> 55

aagcttgccc aggaaccact agtgctcaca cagctctgcc cacaggggaa acctaaccat 60
gcctgccccc tactcagcag gaaggctctg aagctctgag aggattttga acaagttact 120
gtcacagtga gacagctcgg gctaccatgt aagaaaagct caa 163

<210> 56
<211> 45
<212> DNA
<213> mus musculus

<400> 56

tactttgact actggggcca aggcaccact ctcacagtct cctca 45

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

<400> 57

gaggtccagc tggtacagtc tggggctgag gtgaagaagc ctggggctac agtgaaaatc 60
tcctgcaagg tttctggata caccttcacc gactactaca tgcactgggt gcaacaggcc 120
cctggaaaag ggcttgagtg gatgggactt gttgatcctg aagatggtga aacaatatac 180
gcagagaagt tccagggcag agtcaccata accgcggaca cgtctacaga cacagcctac 240
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<210> 58
<211> 17
<212> DNA
<213> homo sapiens

<400> 58

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

<400> 59

tactttgact actggggcca aggaaccctg gtcaccgtct cctcag 46

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

<400> 60

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cccggacaaa ggcttgagtg gatgggatgg atcaacgctg gcaatggtaa cacaaaatat 180
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<210> 61
<211> 299
<212> PRT
<213> homo sapiens

<400> 61

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1 5 10 15
Leu Ala Ile Leu Leu Cys Ser Leu Ala Leu Gly Ser Val Thr Val His

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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
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<210> 62
<211> 897
<212> DNA
<213> homo sapiens

<400> 62

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cctgagaata atcctgtgaa gttgtcctgt gcctactcgg gcttttcttc tccccgtgtg    180
gagtggaagt ttgaccaagg agacaccacc agactcgttt gctataataa caagatcaca    240
gcttcctatg aggaccgggt gaccttcttg ccaactggta tcaccttcaa gtccgtgaca    300
cgggaagaca ctgggacata cacttgtatg gtctctgagg aaggcggcaa cagctatggg    360
gaggtcaagg tcaagctcat cgtgcttgtg cctccatcca agcctacagt taacatcccc    420
tcctctgcca ccattgggaa ccgggcagtg ctgacatgct cagaacaaga tggttcccca    480
ccttctgaat acacctggtt caaagatggg atagtgatgc ctacgaatcc caaaagcacc    540
cgtgccttca gcaactcttc ctatgtcctg aatcccacaa caggagagct ggtctttgat    600
cccctgtcag cctctgatac tggagaatac agctgtgagg cacggaatgg gtatgggaca    660
cccatgactt caaatgctgt gcgcatggaa gctgtggagc ggaatgtggg ggtcatcgtg    720
gcagccgtcc ttgtaaccct gattctcctg ggaatcttgg tttttggcat ctggtttgcc    780
tatagccgag gccactttga cagaacaaag aaagggactt cgagtaagaa ggtgatttac    840
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<210> 63
<211> 259
<212> PRT
<213> homo sapiens

<400> 63

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

<400> 64

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cctgagaata atcctgtgaa gttgtcctgt gcctactcgg gcttttcttc tccccgtgca    120
gcttcctatg aggaccgggt gaccttcttg ccaactggta tcaccttcaa gtccgtgaca    180
cgggaagaca ctgggacata cacttgtatg gtctctgagg aaggcggcaa cagctatggg    240
gaggtcaagg tcaagctcat cgtgcttgtg 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 cacggaatgg gtatgggaca    540
cccatgactt caaatgctgt gcgcatggaa gctgtggagc ggaatgtggg ggtcatcgtg    600
gcagccgtcc ttgtaaccct gattctcctg ggaatcttgg tttttggcat ctggtttgcc    660
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tatagccgag gccactttga cagaacaaag aaagggactt cgagtaagaa ggtgatttac    720
agccagccta gtgcccgaag tgaaggagaa ttcaaacaga cctcgtcatt cctggtg      777
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