NOVEL ANTIBODIES USED TO TREAT CANCER

The present invention relates to new antibodies, especially monoclonal antibodies of murine origin, which are chimeric and humanised, and which are capable of inhibiting tumour growth, and also to the amino acid and nucleic sequences coding for those antibodies. According to a particular aspect, the invention relates to new antibodies, derivative compounds or functional fragments, which are capable of inhibiting the proliferation of tumour cells. The invention also includes use of those antibodies as medicaments for the prophylactic and/or therapeutic treatment of cancers and also in cancer diagnostic methods or kits. Finally, the invention includes products and/or compositions comprising such antibodies in association, for example, with anti-cancer agents and/or antibodies or conjugated with toxins, and use thereof in the prevention and/or treatment of certain cancers.

CD151, also referred to as PETA-3 or SFA-1, is a membrane protein belonging to the tetraspanin family (Boucheix and Rubinstein, 2001, Cell Mol. Life Sci. 58, 1189-1205; Hemler, 2001, J. Cell Biol. 155, 1103-1107). In humans, CD151 has 253 amino acids and includes 4 membrane fragments and 2 extracellular domains EC1 (18 amino acids, sequence [40-57]) and EC2 (109 amino acids, sequence [113-221]) which are also referred to as extracellular loops. It is to be noted, however, that, in the nucleotide sequence, two variants of CD151 have been identified hitherto, namely one having nucleotides A and C at positions 395 and 409, respectively, [Fitter *et al.*, 1995, Blood 86(4), 1348-1355] and the other having, at the same positions, nucleotides G and T instead of nucleotides A and C [Hasegawa *et al.*, 1996, J. Virol. 70(5), 3258-3263]. As a result, a mutation can be observed in the peptide sequence, namely a mutation of the residues K (Lys) and P (Pro) at positions 132 and 137, respectively, to the residues R (Arg) and S (Ser) [Fitter *et al.*, 1995, Blood 86(4), 1348-1355 / Hasegawa *et al.*, 1996, J. Virol. 70(5), 3258-3263].

CD151 is overexpressed in numerous cancers such as, for example, cancers of the lung [Tokuhara *et al.*, 2001, Clin. Cancer Res. 7, 4109-4114], colon [Hashida *et al.*, 2003, Br. J. Cancer 89, 158-167], prostate [Ang *et al.*, 2004, Cancer Epidemiol.

Biomarkers Prev. 13, 1717-1721] or pancreas [Gesierich *et al.*, 2005, Clin. Cancer Res. 11, 2840-2852].

The use of knock-out mice which do not express CD151 and of anti-CD151 antibodies and siRNA in order to block, *in vitro*, the functionality and expression of CD151 in various types of cell has allowed it to be shown that CD151 is involved in a number of phenomena related to cancer, such as cell adhesion (Nishiuchi *et al.*, 2005, Proc. Natl. Acad. Sci. USA 102, 1939-1944; Winterwood *et al.*, 2006, Mol. Biol. Cell 17, 2707-2721), cell motility (Kohno *et al.*, 2002, Int. J. Cancer 97, 336-343), cell migration (Yauch *et al.*, 1998, Mol. Biol. Cell 9, 2751-2765; Testa *et al.*, 1999, Cancer Res. 59, 3812-3820; Penas *et al.*, 2000, J. Invest. Dermatol. 114, 1126-1135; Klosek *et al.*, 2005, Biochem. Biophys. Res. Commun. 336, 408-416), cell invasion (Kohno *et al.*, 2002, Int. J. Cancer 97, 336-343; Shiomi *et al.*, 2005, Lab. Invest. 85, 1489-1506; Hong *et al.*, 2006, J. Biol. Chem. 281, 24279-24292) and angiogenesis (Yanez-Mo *et al.*, 1998, J. Cell Biol. 141, 791-804; Sincock *et al.*, 1999, J. Cell Sci. 112, 833-844; Takeda *et al.*, 2007, Blood 109, 1524-1532).

One of the noteworthy properties of the tetraspanins is their ability to form associations amongst themselves and also with a large number of other surface molecules so as to form structured macromolecular complexes. Within those complexes, each tetraspanin is associated specifically with one or more surface molecules, thereby forming primary complexes composed of a tetraspanin and a partner molecule. The tetraspanins are capable of organising particular microdomains of the plasma membrane from which microdomains they may recruit their partner molecules, which may be functionally coupled. The set of interactions involving the tetraspanins has been referred to as the "network of tetraspanins" or "Tetraspanin Web".

CD151 interacts on the surface of cells with various membrane proteins. In particular, there have been identified highly stable complexes, resistant to the action of certain detergents, with laminin receptor integrins, more particularly with the integrins $\alpha 3\beta 1$ or $\alpha 6\beta 4$, whose preferred ligand is laminin 5 (Yauch *et al.*, 1998, Mol. Biol. Cell 9, 2751-2765; Lammerding *et al.*, 2003, Proc. Natl. Acad. Sci USA 100, 7616-7621). This association involves the extracellular domains of CD151 and the integrins. The sequence QRD [194-196] of CD151, located in the EC2 loop, is very important in that

association because mutation of this site causes loss of interaction with certain integrins (Kazarov *et al.*, 2002, J. Cell Biol. 158, 1299-1309). Functional ternary complexes of CD151/integrin α6β4/c-Met (HGF receptor) have moreover been identified in tumour cells (Klosek *et al.*, 2005, Biochem. Biophys. Res. Commun. 336, 408-416). Inhibition of the expression of CD151 as a result of treating cells with an interference RNA results in inhibition of the cell growth and migration caused by HGF.

The interactions, within a particular cell, between CD151 and other tetraspanins, necessary for formation of the network of tetraspanins, are thought to depend on the membrane and cytoplasmic regions of CD151 because it has been shown that deletion of the EC2 loop does not disrupt the association of CD151 with other tetraspanins (Berditchevski, 2001, J. Cell Sci. 114, 4143-4151).

CD151 is capable of regulating the phenomena of cell adhesion, migration and invasion by modulation of various signalling pathways such as, for example, the phosphoinositide pathway *via* an association with PI4-kinase (Yauch *et al.*, 1998, Mol. Biol. Cell 9, 2751-2765), the c-Jun signalling pathway *via* the phosphorylation of FAK, Src, p38-MAPK and JNK (Hong *et al.*, 2006, J. Biol. Chem. 281, 24279-24292), the phosphorylation of integrins by PKC (Zhang *et al.*, 2001, J. Biol. Chem. 276, 25005-25013) and the activation of GTPases of the Rho family (Shigeta *et al.*, 2003, J. Cell Biol. 163, 165-176).

Homophilic-type interactions between cells are also responsible for an increase in cell motility and in expression of the metalloproteinase MMP-9 (Hong *et al.*, 2006, J. Biol. Chem. 281, 24279-24292). Those intercellular CD151-CD151 interactions bring about the activation of c-Jun *via* the phosphorylation of FAK, Src, p38-MAPK and JNK.

Despite the interest in the CD151 protein, two therapeutically aimed antibodies have been generated to date, namely the monoclonal antibodies 50-6 and SFA1.2B4. These 2 antibodies have comparable activities. Although they do inhibit the formation of metastases *in vivo* in animal models, no effect on tumour growth *in vivo* has been established.

The monoclonal antibody 50-6 (IgG1 isotype) directed to CD151 was generated in the mouse by subtractive immunisations with human epidermoid carcinoma HEp-3 cells (Testa *et al.*, 1999, Cancer Res. 59, 3812-3820).

The antibody 50-6 is capable of inhibiting, *in vitro*, migration of human cervical carcinoma HeLa cells, transfected so as to overexpress CD151, and of HEp-3 cells and angiogenesis in a model of chorio-allantoic membrane neovascularisation caused by bFGF (basic fibroblast growth factor). *In vivo* it inhibits the metastases brought about by inoculation of HEp-3 cells in 2 chicken embryo models (Testa *et al.*, 1999, Cancer Res. 59, 3812-3820). In these models, the inhibitory activity of the antibody 50-6 is determined by measurement of the activity of the protein huPA (human urokinase-type plasminogen activator) in lung extracts. According to the authors, this assay reflects the presence of human cells in the lungs. After assaying, the reduction in metastases (dissemination of HEp-3 cells into the chicken embryo lungs) that is brought about by the antibody 50-6 is estimated, by comparison with a control antibody, to be 74% in a so-called "spontaneous metastasis" model, in which inoculation of the cells is followed by injection of the antibody, and 57% in a so-called "experimental metastasis" model, in which the cells and the antibody are inoculated together. According to the authors, the anti-tumour properties of the antibody 50-6 that are observed *in vivo* do not seem to be related to a cytostatic or cytotoxic effect because the antibody showed no effect on the *in vitro* proliferation of HEp-3 cells.

The hybridoma producing the antibody 50-6 is available at the ATCC under the reference CRL-2696 (hybridoma initially deposited under the reference 50-6 [PTA-227]).

The anti-CD151 monoclonal antibody SFA1.2B4 (IgG1 isotype) was generated in the mouse after immunisation by the intraperitoneal route with NIH 3T3 cells transfected by the human CD151 gene (Hasegawa *et al.*, 1996, J. Virol. 70, 3258-3263). The antibody SFA1.2B4 is capable of inhibiting *in vitro* cell invasion and motility of various human tumour lines (Kohno *et al.*, 2002, Int. J. Cancer 97, 336-343). It inhibits *in vivo* the pulmonary metastases caused by colon cancer RPMI14788 and fibrosarcoma HT1080 lines transfected so as to overexpress CD151 (Kohno *et al.*, 2002, Int. J. Cancer 97, 336-343).

Other murine anti-CD151 antibodies have been described in the literature, such as, for example, the monoclonal antibodies 14A2H1 (Ashman *et al.*, 1991, Br. J. Haematol. 79, 263-270; Roberts *et al.*, 1995, Br. J. Haematol. 89, 853-860), TS151 and

TS151R (Serru *et al.*, 1999, Biochem. J. 340, 103-111; Geary *et al.*, 2001, Tissue Antigens 58, 141-153; Charrin *et al.*, J. Biol. Chem. 276, 14329-14337; Chometon *et al.*, 2006, Exp. Cell Res. 312, 983-985). No *in vivo* anti-tumour activity has been described for those various antibodies.

Several experimental studies have shown the major role of the tetraspanins in the formation of metastases by acting either as suppressors or as promoters of metastases. Accordingly, the transfection of tetraspanins such as CD9, CD63 or CD82 reduces the metastatic potential of cancer lines. In contrast, expression of the tetraspanins CD151 and Co-029 seems to produce the opposite effect. These 2 tetraspanins are therefore thought to be promoters of metastasis. These results are consistent with various clinical studies which have shown that, in a number of cancers (breast, lung, oesophagus, stomach, liver, pancreas, colon, prostate, melanoma, ...), CD9 and CD82 are less expressed in primary tumours when there is metastasis and that a reduction in their expression is predictive of a lower survival rate. In lung cancer, the combined reduction in the expression of CD9 and CD82 has been correlated with greater metastatic potential than when expression of just one of those two antigens is reduced.

Several retrospective studies have shown that overexpression of CD151 is associated with aggressiveness of certain cancers, such as lung, colon and prostate cancers, and that it might be considered to be a factor for poor prognosis (Tokuhara *et al.*, 2001, Clin. Cancer Res. 7, 4109-4114; Hashida *et al.*, 2003, Br. J. Cancer 89, 158-167; Ang *et al.*, 2004, Cancer Epidemiol. Biomarkers Prev. 13, 1717-1721). In these cases, mean survival is in fact reduced in those patients having tumours which express CD151, compared to those having tumours which do not express CD151.

The overexpression of CD151 in various human tumor lines (HeLa, RPMI14788, A172, HT1080), brought about by transfection of the corresponding gene, causes an increase in motility, migration and invasion of the transfected cells (Testa *et al.*, 1999, Cancer Res. 59, 3812-3820; Kohno *et al.*, 2002, Int. J. Cancer 97, 336-343). These phenomena are inhibited in the presence of anti-CD151 antibodies.

In accordance with one embodiment, the invention relates to an isolated antibody, or one of its derivative compounds or functional fragments, capable of binding to the CD151 protein, said antibody being characterised in that it comprises at least one CDR

selected from the CDRs of sequence SEQ ID No. 1-6, 17-22, 33-35, 43-48, 59-63, 69-73, 79-81 or 85-89 or at least one CDR whose sequence has at least 80%, preferably 85%, 90%, 95% and 98% identity, after optimal alignment, with the sequences SEQ ID No. 1-6, 17-22, 33-35, 43-48, 59-63, 69-73, 79-81 or 85-89.

In a first aspect, there is provided an isolated antibody, or one of its derivative compounds or functional fragments, capable of binding to the CD151 protein and to inhibit the tumour growth *in vivo*, said antibody being selected in the group consisting of: a) an antibody, or one of its derivative compounds or functional fragments, comprising: i) a light chain comprising the 3 CDRs, as defined according to IMGT numbering system, CDR-L1 of sequence SEQ ID No. 1, CDR-L2 of sequence SEQ ID No. 2 and CDR-L3 of sequence SEQ ID No. 3, respectively; and ii) a heavy chain comprising the 3 CDRs, as defined according to IMGT numbering system, CDR-H1 of sequence SEQ ID No. 4, CDR-H2 of sequence SEQ ID No. 5 and CDR-H3 of sequence SEQ ID No. 6, respectively; b) an antibody, or one of its derivative compounds or functional fragments, comprising: i) a light chain comprising the 3 CDRs, as defined according to IMGT numbering system, CDR-L1 of sequence SEQ ID No. 17, CDR-L2 of sequence SEQ ID No. 18 and CDR-L3 of sequence SEQ ID No. 19, respectively; and ii) a heavy chain comprising the 3 CDRs, as defined according to IMGT numbering system, CDR-H1 of sequence SEQ ID No. 20, CDR-H2 of sequence SEQ ID No. 21 and CDR-H3 of sequence SEQ ID No. 22, respectively; c) an antibody, or one of its derivative compounds or functional fragments, comprising: i) a light chain comprising the 3 CDRs, as defined according to IMGT numbering system, CDR-L1 of sequence SEQ ID No. 33, CDR-L2 of sequence SEQ ID No. 2 and CDR-L3 of sequence SEQ ID No. 3, respectively; and ii) a heavy chain comprising the 3 CDRs, as defined according to IMGT numbering system, CDR-H1 of sequence SEQ ID No. 4, CDR-H2 of sequence SEQ ID No. 34 and CDR-H3 of sequence SEQ ID No. 35, respectively; and d) an antibody, or one of its derivative compounds or functional fragments, comprising: i) a light chain comprising the 3 CDRs, as defined according to IMGT numbering system, CDR-L1 of sequence SEQ ID No.43, CDR-L2 of sequence SEQ ID No. 44 and CDR-L3 of sequence SEQ ID No. 45, respectively; and ii) a heavy chain comprising the 3 CDRs, as defind according to IMGT numbering system, CDR-H1 of sequence SEQ ID No. 46, CDR-H2 of sequence SEQ ID No. 47 and CDR-H3 of sequence SEQ ID No. 48, respectively.

In a second aspect, there is provided a murine hybridoma capable of secreting an antibody as defined above, said hybridoma being selected in the group consisting of the hybridoma deposited at the CNCM, Institut Pasteur, Paris (France) on 22nd February 2008 under number I-3920; the hybridoma deposited at the CNCM, Institut Pasteur, Paris (France) on 22nd February 2008 under number I-3921; the hybridoma deposited at the CNCM, Institut Pasteur, Paris (France) on 21st February 2008 under number I-3918; and the hybridoma deposited at the CNCM, Institut Pasteur, Paris (France) on 21st February 2008 under number I-3919.

In a third aspect, there is provided an isolated nucleic acid, characterised in that it is selected from the following nucleic acids: a) a DNA or RNA nucleic acid coding for an antibody, or one of its derivative compounds or functional fragments, as defined above; and b) a nucleic acid complementary to a nucleic acid as defined under a).

In a fourth aspect, there is provided a vector comprising a nucleic acid as defined above.

In a fifth aspect, there is provide a cell host comprising a vector as defined above.

In a sixth aspect, there is provided a transgenic animal, with the exception of a human being, comprising a cell as defined above.

In a seventh aspect, there is provided a method of producing an antibody, or one of its derivative compounds or functional fragments, as defined above, characterised in that it comprises the following steps: a) culture of a cell as defined above in a suitable culture medium and under suitable culture conditions; and b) recovery of said antibodies, or a functional fragment thereof, thereby produced, from the culture medium or from said cultured cells.

In an eighth aspect, there is provided an antibody, or one of its derivative compounds or functional fragments, capable of being obtained by a method as defined above.

In a ninth aspect, there is provided a composition comprising, as active ingredient, a compound consisting of an antibody, or one of its functional fragments, as defined above or produced by the hybridoma as defined above.

In a tenth aspect, there is provided a composition as defined above, as a medicament.

In an eleventh aspect, there is provided the use of an antibody, or one of its functional fragments, as defined above or produced by the hybridoma as defined above and/or of a composition as defined above, in the preparation of a medicament intended for the prevention or treatment of cancer.

In a twelfth aspect, there is provided an *in vitro* diagnostic method for diseases having overexpression or underexpression of the CD151 protein, starting from a biological sample in which the abnormal presence of the CD151 protein is suspected, characterised in that said biological sample is placed in contact with an antibody as defined above, or produced by the hybridoma as defined above, it being possible for said antibody, where appropriate, to be labelled.

A "functional fragment" of an antibody according to the invention is understood to refer to, especially, an antibody fragment, such as Fv, scFv (sc standing for "single chain"), Fab, $F(ab')_2$, Fab' or scFv-Fc fragments or diabodies, or any fragment whose half-life may have been increased. Such functional fragments will be described in detail hereinbelow in the present description.

A "derivative compound" of an antibody according to the invention is understood to denote, especially, a binding protein comprising a peptide framework or "scaffold" and at least one of the CDRs of the original antibody in order to preserve its recognition ability. Such derivative compounds are well known to the person skilled in the art and will be described in greater detail hereinbelow in the present description.

More preferably, the invention includes the antibodies, their derivative compounds or their functional fragments, according to the present invention, which are especially chimeric or humanised, that are obtained by genetic recombination or by chemical synthesis.

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

A "monoclonal antibody" is to be understood as an antibody derived from a population of substantially homogeneous antibodies. More especially, the individual antibodies of a population are identical with the exception of a few possible mutations that may be produced naturally and that may be present in minimal amounts. In other words, a monoclonal antibody consists of a homogeneous antibody resulting from the proliferation of just one 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 which is usually characterised by heavy chains of one and the same class and sub-class and light chains of just one type. Monoclonal antibodies are highly specific and are

directed to a single antigen. In addition, in contrast to preparations of polyclonal antibodies which customarily comprise different antibodies directed to different determinants, or epitopes, each monoclonal antibody is directed to a single epitope of the antigen.

It must be understood herein that the invention does not relate to the antibodies in natural form, that is to say they are not taken from their natural environment but rather it has been possible to isolate them or obtain them by purification starting from natural sources, or else obtain them by genetic recombination, or by chemical synthesis, and they may accordingly contain non-natural amino acids as will be described hereinbelow.

More especially, in accordance with a first embodiment of the invention, there is described a first antibody which is characterised in that it comprises:

i) a light chain comprising at least one CDR selected from CDR-L1, CDR-L2 and CDR-L3, wherein

- CDR-L1 is selected from the CDRs of sequence SEQ ID No. 1 or 59, or at least one sequence which has at least 80% identity, after optimal alignment, with sequence SEQ ID No. 1 or 59;

- CDR-L2 is selected from the CDRs of sequence SEQ ID No. 2 or 60, or at least one sequence which has at least 80% identity, after optimal alignment, with sequence SEQ ID No. 2 or 60;

- CDR-L3 is of sequence SEQ ID No. 3 or at least one sequence which has at least 80% identity, after optimal alignment, with the sequence SEQ ID No. 3; and/or

ii) a heavy chain comprising at least one CDR selected from CDR-H1, CDR-H2 and CDR-H3, wherein

- CDR-H1 is selected from the CDRs of sequence SEQ ID No. 4 or 61, or at least one sequence which has at least 80% identity, after optimal alignment, with the sequence SEQ ID No. 4 or 61;

- CDR-H2 is selected from the CDRs of sequence SEQ ID No. 5 or 62, or at least one sequence which has at least 80% identity, after optimal alignment, with the sequence SEQ ID No. 5 or 62;

- CDR-H3 is selected from the CDRs of sequence SEQ ID No. 6 or 63, or at least one sequence which has at least 80% identity, after optimal alignment, with the sequence SEQ ID No. 6 or 63.

In accordance with a second embodiment of the invention, there is described a second antibody which is characterised in that it comprises:

i) a light chain comprising at least one CDR selected from CDR-L1, CDR-L2 and CDR-L3, wherein

- CDR-L1 is selected from the CDRs of sequence SEQ ID No. 17 or 69, or at least one sequence which has at least 80% identity, after optimal alignment, with the sequence SEQ ID No. 17 or 69;

- CDR-L2 is selected from the CDRs of sequence SEQ ID No. 18 or 70, or at least one sequence which has at least 80% identity, after optimal alignment, with the sequence SEQ ID No. 18 or 70;

- CDR-L3 is of sequence SEQ ID No. 19 or at least one sequence which has at least 80% identity, after optimal alignment, with the sequence SEQ ID No. 19; and/or

ii) a heavy chain comprising at least one CDR selected from CDR-H1, CDR-H2 and CDR-H3, wherein

- CDR-H1 is selected from the CDRs of sequence SEQ ID No. 20 or 71, or at least one sequence which has at least 80% identity, after optimal alignment, with the sequence SEQ ID No. 20 or 71;

- CDR-H2 is selected from the CDRs of sequence SEQ ID No. 21 or 72, or at least one sequence which has at least 80% identity, after optimal alignment, with the sequence SEQ ID No. 21 or 72;

- CDR-H3 is selected from the CDRs of sequence SEQ ID No. 22 or 73, or at least one sequence which has at least 80% identity, after optimal alignment, with the sequence SEQ ID No. 22 or 73.

In accordance with a further, third embodiment of the invention, there is described a third antibody which is characterised in that it comprises:

i) a light chain comprising at least one CDR selected from CDR-L1, CDR-L2 and CDR-L3, wherein

- CDR-L1 is selected from the CDRs of sequence SEQ ID No. 33 or 79, or at least one sequence which has at least 80% identity, after optimal alignment, with the sequence SEQ ID No. 33 or 79;

- CDR-L2 is selected from the CDRs of sequence SEQ ID No. 2 or 60, or at least one sequence which has at least 80% identity, after optimal alignment, with the sequence SEQ ID No. 2 or 60;

- CDR-L3 is of sequence SEQ ID No. 3 or at least one sequence which has at least 80% identity, after optimal alignment, with the sequence SEQ ID No. 3; and/or

ii) a heavy chain comprising at least one CDR selected from CDR-H1, CDR-H2 and CDR-H3, wherein

- CDR-H1 is selected from the CDRs of sequence SEQ ID No. 4 or 61, or at least one sequence which has at least 80% identity, after optimal alignment, with the sequence SEQ ID No. 4 or 61;

- CDR-H2 is selected from the CDRs of sequence SEQ ID No. 34 or 80, or at least one sequence which has at least 80% identity, after optimal alignment, with the sequence SEQ ID No. 34 or 80;

- CDR-H3 is selected from the CDRs of sequence SEQ ID No. 35 or 81, or at least one sequence which has at least 80% identity, after optimal alignment, with the sequence SEQ ID No. 35 or 81.

Lastly, in accordance with a final embodiment of the invention, there is described a fourth antibody which is characterised in that it comprises:

i) a light chain comprising at least one CDR selected from CDR-L1, CDR-L2 and CDR-L3, wherein

- CDR-L1 is selected from the CDRs of sequence SEQ ID No. 43 or 85, or at least one sequence which has at least 80% identity, after optimal alignment, with the sequence SEQ ID No. 43 or 85;

- CDR-L2 is selected from the CDRs of sequence SEQ ID No. 44 or 86, or at least one sequence which has at least 80% identity, after optimal alignment, with the sequence SEQ ID No. 44 or 86;

- CDR-L3 is of sequence SEQ ID No. 45 or at least one sequence which has at least 80% identity, after optimal alignment, with the sequence SEQ ID No. 45; and/or

ii) a heavy chain comprising at least one CDR selected from CDR-H1, CDR-H2 and CDR-H3, wherein

- CDR-H1 is selected from the CDRs of sequence SEQ ID No. 46 or 87, or at least one sequence which has at least 80% identity, after optimal alignment, with the sequence SEQ ID No. 46 or 87;

- CDR-H2 is selected from the CDRs of sequence SEQ ID No. 47 or 88, or at least one sequence which has at least 80% identity, after optimal alignment, with the sequence SEQ ID No. 47 or 88;

- CDR-H3 is selected from the CDRs of sequence SEQ ID No. 48 or 89, or at least one sequence which has at least 80% identity, after optimal alignment, with the sequence SEQ ID No. 48 or 89.

In general manner it is recalled here that a CDR region or CDR is understood to denote the hypervariable regions of the heavy chains and light chains of immunoglobulins. There are 3 heavy-chain CDRs and 3 light-chain CDRs. The term CDR or CDRs is used herein to denote, depending on the particular case, one of those regions or a plurality of, or indeed the entirety of, those regions which contain the majority of the amino acid residues responsible for the binding affinity of the antibody for the antigen or the epitope which it recognises.

According to a first approach, the CDRs can be defined in accordance with the IMGT system. The IMGT unique numbering system was defined so as to compare variable domains whatever the antigen, chain type or 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 this numbering system, the conserved amino acids always retain the same position, such as cysteine 23 (1st-CYS), tryptophan 41 (CONSERVED TRP), the hydrophobic amino acid 89, cysteine 104 (2nd-CYS), phenylalanine or tryptophan 118 (J-PHE or TRP). The IMGT unique numbering system accordingly provides standardised delimitation of the scaffold regions (FR1-IMGT: positions 1 to 26, FR2-IMGT: positions 39 to 55, FR3-IMGT: positions 66 to 104 and FR4_IMGT: positions 118 to 128) and also of the complementarity determining regions or CDRs (CDR1-IMGT: positions 27 to 38,

CDR2-IMGT: positions 56 to 65 and CDR3-IMGT: positions 105 to 117). As the "holes" or "spaces" represent unoccupied positions, the lengths of the CDRs according to IMGT become crucial information. The IMGT system is used in 2D graphical representations which are then referred to as IMGT pearl necklaces [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 referred to as 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)].

In accordance with this first approach, the first antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a light chain comprising at least one CDR selected from CDR-L1, CDR-L2 and CDR-L3 of sequence SEQ ID Nos. 1, 2 and 3, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID No. 1, 2 and 3; and/or a heavy chain comprising at least one CDR selected from CDR-H1, CDR-H2 and CDR-H3 of sequence SEQ ID Nos. 4, 5 and 6, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 4, 5 and 6.

For more clarity, the protein sequences mentioned are the following:

SEQ ID No. 1: ASVEYYGTSL

SEQ ID No. 2: EAS

SEQ ID No. 3: QQSRKAPYT

SEQ ID No. 4: GFTFSTYT

SEQ ID No. 5: ISSGGGTT

SEQ ID No. 6: ATPRIGTGFAY

More especially, in preferred manner, the antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a light chain comprising the three CDRs, as defined according to IMGT, of sequence SEQ ID Nos. 1, 2 and 3, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 1, 2 and 3.

In likewise preferred manner, the antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a heavy chain comprising the three CDRs, as defined according to IMGT, of sequence SEQ ID Nos. 4, 5 and 6, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 4, 5 and 6.

In accordance with this same approach, the second antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a light chain comprising at least one CDR selected from CDR-L1, CDR-L2 and CDR-L3 of sequence SEQ ID Nos. 17, 18 and 19, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 17, 18 and 19; and/or a heavy chain comprising at least one CDR selected from CDR-H1, CDR-H2 and CDR-H3 of sequence SEQ ID Nos. 20, 21 and 22, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 20, 21 and 22.

For more clarity, the protein sequences mentioned are the following:

SEQ ID No. 17: ATPRIGTGFAY

SEQ ID No. 18: SAS

SEQ ID No. 19: QQYSSNPT

SEQ ID No. 20: GFTLSTSGMG

SEQ ID No. 21: IYWDDDK

SEQ ID No. 22: ARRDHYGDYSYAMDY

More especially, in preferred manner, the antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a light chain comprising the three CDRs, as defined according to IMGT, of sequence SEQ ID Nos. 17, 18 and 18, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 17, 18 and 19.

In likewise preferred manner, the antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a heavy chain comprising the three CDRs, as defined according to IMGT, of sequence SEQ ID Nos. 20, 21 and 22,

respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 20, 21 and 22.

Still in accordance with this same approach, the third antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a light chain comprising at least one CDR selected from CDR-L1, CDR-L2 and CDR-L3 of sequence SEQ ID Nos. 33, 2 and 3, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 33, 2 and 3; and/or a heavy chain comprising at least one CDR selected from CDR-H1, CDR-H2 and CDR-H3 of sequence SEQ ID Nos. 4, 34 and 35, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 4, 34 and 35.

For more clarity, the protein sequences mentioned are the following:

SEQ ID No. 33: ASVDYYGTSL

SEQ ID No. 34: ISSGGVTT

SEQ ID No. 35: TSPRTGTGFAY

More especially, in preferred manner, the antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a light chain comprising the three CDRs, as defined according to IMGT, of sequence SEQ ID Nos. 33, 2 and 3, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 33, 2 and 3.

In likewise preferred manner, the antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a heavy chain comprising the three CDRs, as defined according to IMGT, of sequence SEQ ID Nos. 4, 34 and 35, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 4, 34 and 35.

Finally, still in accordance with this same approach, the fourth and last antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a light chain comprising at least one CDR selected from CDR-L1, CDR-L2 and CDR-L3 of sequence SEQ ID Nos. 43, 44 and 45, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the

sequences SEQ ID Nos. 43, 44 and 45; and/or a heavy chain comprising at least one CDR selected from CDR-H1, CDR-H2 and CDR-H3 of sequence SEQ ID Nos. 46, 47 and 48, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 46, 47 and 48.

For more clarity, the protein sequences mentioned are the following:

SEQ ID No. 43: ENVGTY

SEQ ID No. 44: GAS

SEQ ID No. 45: GQTYSFPYT

SEQ ID No. 46: GYTFTSSS

SEQ ID No. 47: IHPNSGNT

SEQ ID No. 48: ARARSFYYAMDC

More especially, in preferred manner, the antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a light chain comprising the three CDRs, as defined according to IMGT, of sequence SEQ ID Nos. 43, 44 and 45, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 43, 44 and 45.

In likewise preferred manner, the antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a heavy chain comprising the three CDRs, as defined according to IMGT, of sequence SEQ ID Nos. 46, 47 and 48, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 46, 47 and 48.

According to a second approach, the CDRs can be defined according to the customary approach of 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).

In accordance with this first approach, the first antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a light chain comprising at least one CDR selected from CDR-L1, CDR-L2 and CDR-L3 of sequence SEQ ID Nos. 59, 60 and 3, respectively, or having at least 80%, preferably 85%, 90%,

95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 59, 60 and 3; and/or a heavy chain comprising at least one CDR selected from CDR-H1, CDR-H2 and CDR-H3 of sequence SEQ ID Nos. 61, 62 and 63, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 61, 62 and 63.

For more clarity, the protein sequences mentioned are the following:

SEQ ID No. 59: RASASVEYYGTSLMH

SEQ ID No. 60: EASNVES

SEQ ID No. 61: STYTMS

SEQ ID No. 62: YISSGGGTTYYPDTVKG

SEQ ID No. 63: PRIGTGFAY

More especially, in preferred manner, the antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a light chain comprising the three CDRs, as defined according to Kabat, of sequence SEQ ID Nos. 59, 60 and 3, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 59, 60 and 3.

In likewise preferred manner, the antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a heavy chain comprising the three CDRs, as defined according to Kabat, of sequence SEQ ID Nos. 61, 62 and 63, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 61, 62 and 63.

In accordance with this same approach, the second antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a light chain comprising at least one CDR selected from CDR-L1, CDR-L2 and CDR-L3 of sequence SEQ ID Nos. 69, 70 and 19, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 69, 70 and 19; and/or a heavy chain comprising at least one CDR selected from CDR-H1, CDR-H2 and CDR-H3 of sequence SEQ ID Nos. 71, 72 and 73, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 71, 72 and 73.

For more clarity, the protein sequences mentioned are the following:

SEQ ID No. 69: KASQNVGIAVA

SEQ ID No. 70: SASNRYT

SEQ ID No. 71: STSGMGVS

SEQ ID No. 72: HIYWDDDKRYNPSLKS

SEQ ID No. 73: RDHYGDYSYAMDY

More especially, in preferred manner, the antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a light chain comprising the three CDRs, as defined according to Kabat, of sequence SEQ ID Nos. 69, 70 and 19, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 69, 70 and 19.

In likewise preferred manner, the antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a heavy chain comprising the three CDRs, as defined according to Kabat, of sequence SEQ ID Nos. 71, 72 and 73, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 71, 72 and 73.

Still in accordance with this same approach, the third antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a light chain comprising at least one CDR selected from CDR-L1, CDR-L2 and CDR-L3 of sequence SEQ ID Nos. 79, 60 and 3, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 79, 60 and 3; and/or a heavy chain comprising at least one CDR selected from CDR-H1, CDR-H2 and CDR-H3 of sequence SEQ ID Nos. 61, 80 and 81, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 100, 101 and 102.

For more clarity, the protein sequences mentioned are the following:

SEQ ID No. 79: ASVDYYGTSL

SEQ ID No. 80: YISSGGVTTYYPDTIKG

SEQ ID No. 81: PRTGTGFAY

More especially, in preferred manner, the antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a light chain comprising the three CDRs, as defined according to Kabat, of sequence SEQ ID Nos. 79, 60 and 3, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 79, 60 and 3.

In likewise preferred manner, the antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a heavy chain comprising the three CDRs, as defined according to Kabat, of sequence SEQ ID No. 61, 80 and 81, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 61, 80 and 81.

Finally, still according to this same approach, the fourth and final antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a light chain comprising at least one CDR selected from CDR-L1, CDR-L2 and CDR-L3 of sequence SEQ ID Nos. 85, 86 and 45, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 85, 86 and 45; and/or a heavy chain comprising at least one CDR selected from CDR-H1, CDR-H2 and CDR-H3 of sequence SEQ ID Nos. 87, 88 and 89, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 87, 88 and 89.

For more clarity, the protein sequences mentioned are the following:

SEQ ID No. 85: KASENVGTYVS

SEQ ID No. 86: GASNRYTGVPD

SEQ ID No. 87: TSSSMH

SEQ ID No. 88: EIHPNSGNTNNNEKFKG

SEQ ID No. 89: ARSFYYAMDC

More especially, in preferred manner, the antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a light chain comprising the three CDRs, as defined according to Kabat, of sequence SEQ ID Nos. 85, 86 and 45, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 85, 86 and 45.

In likewise preferred manner, the antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a heavy chain comprising the three CDRs, as defined according to Kabat, of sequence SEQ ID Nos. 87, 88 and 89, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 87, 88 and 89.

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

It must be understood herein that the invention does not relate to the antibodies in natural form, that is to say they are not taken from their natural environment but may have been isolated or obtained by purification starting from natural sources, or else obtained by genetic recombination, or by chemical synthesis, and they may accordingly contain non-natural amino acids as will be described hereinbelow.

"Percentage identity" between two nucleic acid or amino acid sequences is understood by the present invention to denote the percentage of nucleotides or amino acid residues that are identical between the two sequences being compared, obtained after the best alignment (optimal alignment), this percentage being purely statistical and the differences between the two sequences being randomly distributed throughout their length. Sequence comparisons between two nucleic acid or amino acid sequences are customarily carried out by comparing those sequences after they have been optimally aligned, it being possible for said comparison to be carried out segment by segment or by means of a "comparison window". The optimal alignment of sequences for the comparison can, besides being carried out manually, be carried out 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 employing those 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 N 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 be compared can contain additions or deletions with respect to the reference sequence for optimal alignment between those two sequences. The percentage identity is calculated by determining the number of identical positions where the nucleotide or amino acid residue is identical between the two sequences, dividing that number of identical positions by the total number of positions in the comparison window and multiplying the result obtained by 100 in order to obtain the percentage identity between the two sequences.

For example, there may be used the BLAST program "BLAST 2 sequences" (Tatusova *et al.*, "Blast 2 sequences - a new tool for comparing protein and nucleotide sequences", FEMS Microbiol Lett. 174:247-250) available on the website http://www.ncbi.nlm.nih.gov/gorf/bl2.html, the parameters used being those given as default (especially for the parameters "open gap penalty": 5, and "extension gap penalty": 2; the selected matrix being, for example, the "BLOSUM 62" matrix suggested by the program), the percentage identity between the two sequences for comparison being calculated directly by the program.

As an amino acid sequence having at least 80%, preferably 85%, 90%, 95% and 98%, identity with a reference amino acid sequence preference is given to one having, with respect to the reference sequence, certain modifications, especially a deletion, addition or substitution of at least one amino acid, a truncation or an extension. In the case of a substitution of one or more consecutive or non-consecutive amino acid(s) preference is given to substitutions in which the substituted amino acids are replaced by "equivalent" amino acids. The expression "equivalent amino acids" is intended herein to denote any amino acid capable of being substituted for one of the amino acids of the basic structure without, however, fundamentally modifying the biological activities of the corresponding antibodies, such as will be defined hereinbelow, especially in the Examples.

These equivalent amino acids can be determined either on the basis of their structural homology with the amino acids for which they are being substituted or on the

basis of results of comparative biological activity tests between the various antibodies that may be produced.

By way of non-limiting example, Table 1 below recalls the substitution possibilities capable of being carried out without resulting in fundamental modification of the biological activity of the corresponding modified antibody, the reverse substitutions being feasible of course 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 recognised by the person skilled in the art, as prior art, that the greatest variability (length and composition) between the 6 CDRs is located in the 3 CDRs of the

heavy chain and, more especially, the CDR-H3 of that heavy chain. Consequently, it will be evident that the preferred characteristic CDRs of the antibody according to the invention will be the 3 CDRs of the heavy chain and, even more preferably, the CDR corresponding to the CDR-H3.

In a particular embodiment, the present invention relates to a murine antibody, or one of its derivative compounds or functional fragments, according to the invention.

According to another embodiment of the invention, said first antibody, or one of its derivative compounds or functional fragments, comprises a light chain comprising the three CDRs, as defined according to IMGT, of sequence SEQ ID Nos. 1, 2 and 3, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 1, 2 and 3; and a heavy chain comprising the three CDRs of sequence SEQ ID Nos. 4, 5 and 6, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 4, 5 and 6.

According to yet another embodiment of the invention, said first antibody, or one of its derivative compounds or functional fragments, comprises a light chain comprising the three CDRs, as defined according to Kabat, of sequence SEQ ID Nos. 59, 60 and 3, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 59, 60 and 3; and a heavy chain comprising the three CDRs of sequence SEQ ID Nos. 61, 62 and 63, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 61, 62 and 63.

According to yet another embodiment, the antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a light chain having a sequence comprising the amino acid sequence SEQ ID No. 7 or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequence SEQ ID No. 7, and/or it comprises a heavy chain having a sequence comprising the amino acid sequence SEQ ID No. 8 or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequence SEQ ID No. 8.

For more clarity, the protein sequences mentioned are the following:

SEQ ID No. 7:

DIVLSQSPASLALSLGQRATISCRASASVEYYGTSLMHWYQQKPGQPPKLLIYEA SNVESGVPARFSGSGSGTDFSLNIHPVEEDDLAIYFCQQSRKAPYTFGGGTKLEI K

SEQ ID No. 8:

EVKLVESGGGLVQPGGSLKLSCAASGFTFSTYTMSWVRQTPEKRLEWVAYISS GGGTTYYPDTVKGRFTISRDNARNTLYLQMNSLKSEDTAMYYCATPRIGTGFA YWGQGTLVTVSA

According to another embodiment of the invention, said second antibody, or one of its derivative compounds or functional fragments, comprises a light chain comprising the three CDRs, as defined according to IMGT, of sequence SEQ ID Nos. 17, 18 and 19, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 17, 18 and 19; and a heavy chain comprising the three CDRs of sequence SEQ ID Nos. 20, 21 and 22, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 20, 21 and 22.

According to yet another embodiment of the invention, said second antibody, or one of its derivative compounds or functional fragments, comprises a light chain comprising the three CDRs, as defined according to Kabat, of sequence SEQ ID Nos. 69, 70 and 19, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 69, 70 and 19; and a heavy chain comprising the three CDRs of sequence SEQ ID Nos. 71, 72 and 73, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 71, 72 and 73.

According to yet another embodiment, the antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a light chain having a sequence comprising the amino acid sequence SEQ ID No. 23 or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequence SEQ ID No. 23, and/or comprises a heavy chain having a sequence comprising the amino acid sequence SEQ ID No. 24 or having at least 80%, preferably

85%, 90%, 95% and 98% identity, after optimal alignment, with the sequence SEQ ID No. 24.

For more clarity, the protein sequences mentioned are the following:

SEQ ID No. 23:

DIVMTQSQKFMSTSEGDRVSITCKASQNVGIAVAWYQQKPGQSPKLLIYSASNR YTGVPDRFTGSGSGTDFTLTISNMQSEDLADYFCQQYSSNPTFGAGTKLELK

SEQ ID No. 24:

QVTLKESGPGILQPSQTLSLTCSFSGFTLSTSGMGVSWIRQPSGKGLEWLAHIYW DDDKRYNPSLKSRLTISKDTSRNQVFLKITSVDTADTATYYCARRDHYGDYSYA MDYWGQGTSVTVSS

According to another embodiment of the invention, said third antibody, or one of its derivative compounds or functional fragments, comprises a light chain comprising the three CDRs, as defined according to IMGT, of sequence SEQ ID Nos. 33, 2 and 3, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 33, 2 and 3; and a heavy chain comprising the three CDRs of sequence SEQ ID Nos. 4, 34 and 35, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 4, 34 and 35.

According to yet another embodiment of the invention, said third antibody, or one of its derivative compounds or functional fragments, comprises a light chain comprising the three CDRs, as defined according to Kabat, of sequence SEQ ID Nos. 79, 60 and 3, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 79, 60 and 3; and a heavy chain comprising the three CDRs of sequence SEQ ID Nos. 61, 80 and 81, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 61, 80 and 81.

According to yet another embodiment, the antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a light chain having a sequence comprising the amino acid sequence SEQ ID No. 36 or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with

the sequence SEQ ID No. 36, and/or comprises a heavy chain having a sequence comprising the amino acid sequence SEQ ID No. 37 or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with sequence SEQ ID No. 37.

For more clarity, the protein sequences mentioned are the following:

SEQ ID No. 36:

DIVLTQSPASLALSLGQRATISCRASASVDYYGTSLMQWYQQKPGQPPKLLIYE ASNVESGVPARFSGSGSGTDFSLNIHPVEEDDLAIYFCQQSRKAPYTFGGGTKLE IK

SEQ ID No. 37:

EVKLVESGGDLVQPGGSLKLSCAASGFTFSTYTMSWVRQTPEKRLEWVAYISS GGVTTYYPDTIKGRFTISRDNAKNTLFLQMNSLKSEDTAMYYCTSPRTGTGFAY WGQGTLVTVSA

According to another embodiment of the invention, said fourth antibody, or one of its derivative compounds or functional fragments, comprises a light chain comprising the three CDRs, as defined according to IMGT, of sequence SEQ ID Nos. 43, 44 and 45, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 43, 44 and 45; and a heavy chain comprising the three CDRs of sequence SEQ ID Nos. 46, 47 and 48, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 46, 47 and 48.

According to yet another embodiment of the invention, said fourth antibody, or one of its derivative compounds or functional fragments, comprises a light chain comprising the three CDRs, as defined according to Kabat, of sequence SEQ ID Nos. 85, 86 and 45, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 85, 86 and 45; and a heavy chain comprising the three CDRs of sequence SEQ ID Nos. 87, 88 and 89, respectively, or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequences SEQ ID Nos. 87, 88 and 89.

According to yet another embodiment, the antibody, or one of its derivative compounds or functional fragments, according to the invention comprises a light chain having a sequence comprising the amino acid sequence SEQ ID No. 49 or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequence SEQ ID No. 49, and/or comprises a heavy chain having a sequence comprising the amino acid sequence SEQ ID No. 50 or having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with the sequence SEQ ID No. 50.

For more clarity, the protein sequences mentioned are the following:

SEQ ID No. 49:

NIVMTQSPKSMSMSVGERVTLSCKASENVGTYVSWYQQKPEQSPKLLIYGASN RYTGVPDRFTGSGSATDFTLTISSVQAEDLADYHCGQTYSFPYTFGGGTKLEIK

SEQ ID No. 50:

QVQLQQPGSVLVRPGASVKLSCKASGYTFTSSSMHWAKQRPGQGLEWIGEIHP NSGNTNNNEKFKGKATLTVDTSSSTAYVDLSSLSSEDSAVYYCARARSFYYAM DCWGQGTSVTVSS

As indicated hereinbefore, the invention is likewise directed at any compound derived from an antibody according to the invention.

More especially, the antibody, or one of its derivative compounds or functional fragments, according to the invention is characterised in that said derivative compound consists of a binding protein comprising a peptide scaffold onto which is grafted at least one CDR so as to preserve, in entirety or in part, the paratopic recognition properties of the initial antibody.

One or more sequences among the sequences of the CDRs described in this invention can also be provided on various protein scaffolds - or frameworks - of immunoglobulins. In this case, the protein sequence makes it possible to recreate a peptide skeleton favourable to the folding of the grafted CDR or CDRs, allowing it/them to preserve their paratopic properties of antigen recognition.

In general manner, the person skilled in the art will know how to determine the type of protein scaffold onto which to graft at least one of the CDRs derived from the

original antibody. More especially, it is known that, in order to be selected, such scaffolds must meet the greatest number of criteria as listed below (Skerra A., J. Mol. Recogn. 13, 2000, 167-187):

- good phylogenetic conservation;
- known three-dimensional structure (such as, for example, from crystallography, NMR spectroscopy or any other technique known to the person skilled in the art);
- small size;
- few or no post-transcriptional modifications; and/or
- ease of production, expression and purification.

The origin of such protein scaffolds can be, but is not limited to, structures selected from: fibronectin and preferably the 10th domain of type 3 fibronectin, lipocalin, anticalin (Skerra A., J. Biotechnol., 2001, 74(4) :257-75), protein Z derived from domain B of protein A of *Staphylococcus aureus*, thioredoxin A and also proteins having repeated motifs of the "ankyrin repeat" (Kohl *et al.*, PNAS, 2003, vol.100, No. 4, 1700-1705), "armadillo repeat", "leucine-rich repeat" or "tetratricopeptide repeat" types.

There may also be mentioned scaffolds derived from toxins such as, for example, the following toxins derived from scorpions, insects, plants, molluscs, etc. or protein inhibitors of neuronal NO synthase (PIN).

As an example - which is in no way limiting - of such hybrid structures there may be mentioned the insertion of the CDR-H1 (heavy chain) of an anti-CD4 antibody, namely 13B8.2, into one of the loops of PIN, the new binding protein thereby obtained retaining the same binding properties as the original antibody (Bes *et al.*, BBRC 343, 2006, 334-344). There may also be mentioned, by way of illustration, the grafting of the CDR-H3 (heavy chain) of an anti-lysozyme VHH antibody onto one of the loops of neocarzinostatin (Nicaise *et al.*, 2004).

Finally, as described hereinbefore, such peptide scaffolds can comprise from 1 to 6 CDR(s) derived from the original antibody. Preferably, but without its being a necessity, the person skilled in the art will select at least one CDR derived from the heavy chain, the latter being known to be principally responsible for the specificity of the antibody. Selection of the relevant CDR(s) will be obvious to the person skilled in

the art, the latter employing known techniques for the purpose (Bes *et al.*, FEBS letters 508, 2001 67-74).

Obviously, these examples are in no way limiting, and any other structure known or obvious to the skilled person must be considered as being included within the protection afforded by the present patent application.

The present invention accordingly relates to an antibody, or one of its derivative compounds or functional fragments, characterised in that said peptide scaffold is selected from proteins which a) are phylogenetically well conserved, b) are of robust architecture, c) have well-known three-dimensional molecular organisation, d) are of small size and/or e) comprise regions that can be modified by deletion and/or insertion without changes to the stability properties.

According to a preferred embodiment, the antibody, or one of its derivative compounds or functional fragments, according to the invention is characterised in that the peptide scaffold is selected from i) scaffolds derived from fibronectin, preferably the 10th domain of type 3 fibronectin, lipocalin, anticalin, protein Z derived from domain B of protein A of *Staphylococcus aureus*, thioredoxin A, ii) proteins having repeated motifs of the "ankyrin repeat", "armadillo repeat", "leucine-rich repeat" or "tetratricopeptide repeat" type and also iii) protein inhibitors of neuronal NO synthase (PIN).

In accordance with another aspect of the invention, mention is likewise made of the functional fragments of the antibody described hereinbefore.

More especially, the invention is directed to an antibody, or one of its derivative compounds or functional fragments, the invention characterised in that said functional fragment is selected from Fv, Fab, $(Fab')_2$, Fab', scFv and scFv-Fc fragments and diabodies, and any fragment whose half-life may have been extended such as pegylated fragments.

Such functional fragments of antibodies according to the invention consist, for example, of Fv, scFv (sc standing for single chain), Fab, $F(ab')_2$, Fab' or scFv-Fc fragments or diabodies, or any fragment whose half-life may have been extended by chemical modification, e.g. addition of poly(alkylene)glycol such as poly(ethylene)glycol ("PEGylation") (the PEGylated fragments being referred to as

Fv-PEG, scFv-PEG, Fab-PEG, F(ab')$_2$-PEG or Fab'-PEG) ("PEG" from the designation Poly(Ethylene)Glycol), or by incorporation in a liposome, microspheres or PLGA, said fragments having at least one of the characteristic CDRs according to the invention and, especially, being capable of generally exerting activity, even partial, of the antibody from which it is derived.

Preferably, said functional fragments will be composed of or will comprise 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 is derived and an adequate affinity, preferably equal to at least 1/100th, more preferably at least 1/10th, of that of the antibody from which it is derived.

Such a functional fragment will comprise at least 5 consecutive amino acids, preferably 10, 15, 25, 50 or 100 consecutive amino acids, from the sequence of the antibody from which it is derived.

Preferably, these functional fragments will be fragments of Fv, scFv, Fab, F(ab')$_2$, F(ab'), scFv-Fc type, or diabodies, which generally have the same fixing specificity as the antibody from which they are obtained. According to the present invention, fragments of antibodies of the invention can be obtained starting from antibodies as described hereinbefore by methods such as digestion using enzymes such as pepsin or papain and/or by cleavage of the disulfide bridges by means of chemical reduction. The antibody fragments included in the present invention can also be obtained by genetic recombination techniques that are likewise well-known to the person skilled in the art or by peptide synthesis by means of, for example, automatic peptide synthesisers such as those supplied by the company Applied Biosystems, etc..

According to another particular aspect, the present invention relates to a chimeric antibody, or one of its derivative compounds or functional fragments, according to the invention, characterised in that said antibody also comprises constant light chain and heavy chain regions derived from an antibody from a species heterologous to the mouse, especially from humans.

According to yet another aspect of the invention, the humanised antibody, or one of its derivative compounds or functional fragments, is characterised in that the constant

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

According to another aspect, the invention relates to a first murine hybridoma capable of secreting a monoclonal antibody according to the present invention, especially the hybridoma of murine origin as deposited at the Centre National de Cultures de Microorganismes (CNCM) (Institut Pasteur, Paris, France) on 22nd February 2008 under number I-3920. Said hybridoma was obtained by fusion of immunised Balb/c mice splenocytes and Sp 2O Ag 14 myeloma cell lines. Said hybridoma deposited at the CNCM on 22nd February 2008 under number I-3920 secretes the monoclonal antibody referred to herein as 203B6, or one of its derivative compounds or functional fragments.

According to another aspect, the invention relates to a second murine hybridoma capable of secreting a monoclonal antibody according to the present invention, especially the hybridoma of murine origin as deposited at the Centre National de Cultures de Microorganismes (CNCM) (Institut Pasteur, Paris, France) on 22nd February 2008 under number I-3921. Said hybridoma was obtained by fusion of immunised Balb/c mice splenocytes and Sp 2O Ag 14 myeloma cell lines. Said hybridoma deposited at the CNCM on 22nd February 2008 under number I-3921 secretes the monoclonal antibody referred to herein as 205H8, or one of its derivative compounds or functional fragments.

According to another aspect, the invention relates to a third murine hybridoma capable of secreting a monoclonal antibody according to the present invention, especially the hybridoma of murine origin as deposited at the Centre National de Cultures de Microorganismes (CNCM) (Institut Pasteur, Paris, France) on 21st February 2008 under number I-3918. Said hybridoma was obtained by fusion of immunised Balb/c mice splenocytes and Sp 2O Ag 14 myeloma cell lines. Said hybridoma deposited at the CNCM on 21st February 2008 under number I-3918 secretes the monoclonal antibody referred to herein as 211F3, or one of its derivative compounds or functional fragments.

Finally, according to another aspect, the invention relates to a fourth murine hybridoma capable of secreting a monoclonal antibody according to the present

invention, especially the hybridoma of murine origin as deposited at the Centre National de Cultures de Microorganismes (CNCM) (Institut Pasteur, Paris, France) on 21st February 2008 under number I-3919. Said hybridoma was obtained by fusion of immunised Balb/c mice splenocytes and Sp 2O Ag 14 myeloma cell lines. Said hybridoma deposited at the CNCM on 21st February 2008 under number I-3919 secretes the monoclonal antibody referred to herein as 214B2, or one of its derivative compounds or functional fragments.

For more clarity, Table 2 hereinbelow summarises the different amino acid sequences corresponding to the different antibodies according to the invention, with the CDR sequences defined according to IMGT in bold and those defined according to Kabat in italics.

Table 2

	203B6 (I-3920)		205H8 (I-3921)		211F3 (I-3918)		214B2 (I-3919)	
CDR-L1	**1**	*59*	**17**	*69*	**33**	*79*	**43**	*85*
CDR-L2	**2**	*60*	**18**	*70*	**2**	*60*	**44**	*86*
CDR-L3	**3**	*3*	**19**	*19*	**3**	*3*	**45**	*45*
Light Chain (VL)	7		23		36		49	
Chim. VL	101						95	
CDR-H1	**4**	*61*	**20**	*71*	**4**	*61*	**46**	*87*
CDR-H2	**5**	*62*	**21**	*72*	**34**	*80*	**47**	*88*
CDR-H3	**6**	*63*	**22**	*73*	**35**	*81*	**48**	*89*
Heavy Chain (VH)	8		24		37		50	
IgG1 Chim. VH	102						96	
IgG4 Chim. VH	103						97	

Antibodies according to the present invention also include chimeric or humanised antibodies.

A chimeric (also referred as chimaeric) antibody is understood as referring to an antibody which contains a natural variable (light chain and heavy chain) region derived from an antibody from a given species in association with the constant light chain and heavy chain regions of an antibody from a heterologous species to said given species.

Chimeric-type antibodies, or their fragments, according to the invention can be prepared using genetic recombination techniques. For example, the chimeric antibody may be produced by cloning a recombinant DNA comprising a promoter and a sequence

coding for the variable region of a non-human, especially murine, monoclonal antibody according to the invention and a sequence coding for the constant region of a human antibody. A chimeric antibody of the invention encoded by such a recombinant gene may be, for example, a mouse-human chimaera, the specificity of that antibody being determined by the variable region derived from the murine DNA and its isotype determined by the constant region derived from the human DNA. For methods of preparing chimeric antibodies, reference may be made, for example to the document Verhoeyn *et al.* (BioEssays, 8:74, 1988).

A humanised antibody is understood as referring to an antibody which contains CDR regions derived from an antibody of non-human origin, the other parts of the antibody molecule being derived from one (or more) human antibody/antibodies or germline(s). In addition, some of the residues of the segments of the skeleton (referred to as FR) can be modified in order to preserve the 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 humanised antibodies according to the invention or fragments thereof can be prepared by techniques known to the 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; or Bebbington *et al.*, Bio/Technology, 10:169-175, 1992). Such humanised antibodies according to the invention are preferred for their use in *in vitro* diagnostic methods or in *in vivo* prophylactic and/or therapeutic treatment. Other humanisation techniques are also known to the person skilled in the art, such as, for example, the technique of "*CDR Grafting*", described by PDL, which is the subject-matter of patents EP 0 451 261, EP 0 682 040, EP 0 939 127, EP 0 566 647 or also US 5,530,101, US 6,180,370, US 5,585,089 and US 5,693,761. There may also be mentioned the patents US 5,639,641 or also 6,054,297, 5,886,152 and 5,877,293.

In addition, the invention is also directed at the humanised antibodies derived from the murine and chimeric antibodies described hereinbefore.

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

In the embodiment corresponding to the IgG1 isotype, an additional characteristic of the antibody is that of having effector functions such as ADCC (Antibody Dependent Cellular Cytotoxicity) and/or CDC (Complement Dependent Cytotoxicity).

In addition, as will emerge from the Examples hereinbelow, the antibody to which the present invention relates differs from hitherto known antibodies in that it is capable of inhibiting the proliferation of tumour cells.

As has been stated hereinbefore, the CD151 protein belongs to the tetraspanin family and, by virtue thereof, contains 2 extracellular domains EC1 (18 amino acids, sequence [40-57]) and EC2 (109 amino acids, sequence [113-221]), also referred to as extracellular loops.

According to the present invention, the antibodies used are capable of binding to at least one epitope located in the extracellular domain. Preferably, said antibody will fix itself to the loops EC1 and/or EC2.

More particularly, in accordance with a preferred embodiment of the invention, there is described the use of at least one anti-CD151 antibody, or one of its functional fragments, capable of binding to an epitope included in the extracellular loop 1 (EC1) and/or 2 (EC2), preferably EC2, corresponding to the amino acids 40-57 and 113-221, respectively, of the CD151 protein.

The EC1 loop [40-57] contains 18 amino acids and has a theoretical weight of 2002.2 Da.

The EC2 loop [113-221] has an N-glycosylation site (residue Asn159) and 6 cysteine residues forming 3 disulfide bridges. A structural model of the EC2 loop of the tetraspanins, and especially of CD151, has been proposed on the basis of the three-dimensional structure of the EC2 loop of the tetraspanin CD81 (Seigneuret *et al.*, 2001, J. Biol. Chem. 276, 40055-40064). According to that model, the tetraspanins have a common, relatively conserved scaffold composed of 3 α helices and a specific variable domain. For CD151, that scaffold is thought to be composed of the regions [113-157]

and [209-221], and the variable domain is thought to be composed of the region [158-208].

The variable domain of the EC2 loop is thought to be more especially involved in the specific interactions of CD151 with proteins of the integrin family. Directed mutagenesis experiments have especially shown the importance of the region [193-208], and more precisely of the tripeptide QRD [194-196] and the cysteine residue at position 192, in the association of CD151 with certain laminin receptor integrins such as integrins $\alpha 3\beta 1$ or $\alpha 6\beta 4$ (Kazarov *et al.*, 2002, J. Cell Biol. 158, 1299-1309).

Even more preferably, the present invention is directed at the use of at least one anti-CD151 antibody, or one of its functional fragments, capable of binding to an epitope of the EC2 region.

In a new aspect, the present invention relates to an isolated nucleic acid, characterised in that it is selected from the following nucleic acids:

a) a DNA or RNA nucleic acid coding for an antibody, or one of its derivative compounds or functional fragments, as defined hereinbefore;

b) a nucleic acid complementary to a nucleic acid as defined hereinbefore under a);

c) a nucleic acid of at least 18 nucleotides capable of hybridising under conditions of high stringency with at least one of the nucleic acid sequences SEQ ID No. 9-16, 25-32, 38-42, 51-58, 64-68, 74-78, 82-84, 90-94, 98-100 or 104-106 or with a sequence having at least 80%, preferably 85%, 90%, 95% and 98%, identity, after optimal alignment, with said sequences.

Table 3 hereinbelow summarises the different nucleotide sequences relating to antibodies according to the invention, with the CDR sequences defined according to IMGT in bold and those defined according to Kabat in italics.

Table 3

	203B6 (I-3920)		205H8 (I-3921)		211F3 (I-3918)		214B2 (I-3919)	
CDR-L1	**9**	*64*	**25**	*74*	**38**	*82*	**51**	*90*
CDR-L2	**10**	*65*	**26**	*75*	**10**	*65*	**52**	*91*
CDR-L3	**11**	*11*	**27**	*27*	**11**	*11*	**53**	*53*
Light Chain	15		31		41		57	
CDR-H1	**12**	*66*	**28**	*76*	**12**	*66*	**54**	*92*
CDR-H2	**13**	*67*	**29**	*77*	**39**	*83*	**55**	*93*
CDR-H3	**14**	*68*	**30**	*78*	**40**	*84*	**56**	*94*
Heavy Chain	16		32		42		58	

The terms nucleic acid, nucleic or nucleic acid sequence, polynucleotide, oligonucleotide, polynucleotide sequence, nucleotide sequence, which will be used interchangeably in the present description, are understood as referring to a precise concatenation of nucleotides, modified or not, making it possible to define a fragment or region of a nucleic acid, including or not including non-natural nucleotides, which may correspond equally to a double-stranded DNA, a single-stranded DNA and to transcription products of said DNAs.

It must also be understood herein that the present invention does not relate to the nucleotide sequences in their natural chromosomic environment, that is to say in the natural state. They are sequences which have been isolated and/or purified, that is to say they have been extracted directly or indirectly, for example by copying, their environment having been at least partially modified. They are also understood herein to refer to isolated nucleic acids obtained by genetic recombination using, for example, host cells or obtained by chemical synthesis.

Nucleic sequences having a percentage identity of least 80%, preferably 85%, 90%, 95% and 98%, after optimal alignment, with a preferred sequence are understood to denote the nucleic sequences having, with respect to the reference nucleic sequence, certain modifications such as, especially, a deletion, truncation, extension, chimeric fusion and/or substitution, especially pointwise substitution. They are preferably sequences whose sequences code for the same amino acid sequences as the reference sequence, this being due to the degeneracy of the genetic code, or complementary

sequences which are capable of hybridising specifically with the reference sequences, preferably under conditions of high stringency, especially as defined hereinbelow.

Hybridisation under conditions of high stringency means that the conditions for temperature and ionic strength are so selected that they make it possible for hybridisation to be maintained between two complementary fragments of DNA. By way of illustration, conditions of high stringency of the hybridisation step for the purpose of defining the polynucleotide fragments described hereinbefore are advantageously as follows.

DNA-DNA or DNA-RNA hybridisation is carried out in two steps: (1) prehybridisation at 42°C for 3 hours in phosphate buffer (20mM, pH 7.5) containing 5 x SSC (1 x SSC corresponds to a solution of 0.15M NaCl + 0.015M sodium citrate), 50% formamide, 7% sodium dodecyl sulfate (SDS), 10 x Denhardt's, 5% dextran sulfate and 1% salmon sperm DNA; (2) hybridisation proper for 20 hours at a temperature depending on the size of the probe (i.e.: 42°C for a probe of size > 100 nucleotides) followed by 2 washings of 20 minutes at 20°C in 2 x SSC + 2% SDS, 1 washing of 20 minutes at 20°C in 0.1 x SSC + 0.1% SDS. The final washing is carried out in 0.1 x SSC + 0.1% SDS for 30 minutes at 60°C for a probe of size > 100 nucleotides. The high-stringency conditions described hereinbefore for a polynucleotide of defined size can be adapted by the person skilled in the art for oligonucleotides of larger or smaller size, in accordance with the teaching of Sambrook *et al.*, (1989, Molecular cloning: a laboratory manual. 2nd Ed. Cold Spring Harbor).

The invention relates also to a vector comprising a nucleic acid according to the present invention.

The invention is directed especially at cloning and/or expression vectors containing a nucleotide sequence according to the invention.

The vectors according to the invention preferably comprise elements which allow the expression and/or secretion of the nucleotide sequences in a particular host cell. The vector must then comprise a promoter, translation initiation and termination signals and also appropriate transcription regulation regions. It must be capable of being maintained in stable manner in the host cell and it may optionally have particular signals specifying the secretion of the translated protein. These various elements will be

selected and optimised by the person skilled in the art as a function of the cell host used. To that effect, the nucleotide sequences according to the invention can be inserted into self-replicating vectors within the selected host or can be integrative vectors of the selected host.

Such vectors are prepared by methods customarily used by the 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 according to the invention are, for example, vectors of plasmid or viral origin. They are useful in transforming host cells in order to clone or express the nucleotide sequences according to the invention.

The invention also includes the host cells transformed by or containing a vector according to the invention.

The cell host may be selected from prokaryotic or eukaryotic systems, for example, bacterial cells, but also yeast cells or animal cells, especially mammalian cells. Insect cells or plant cells can also be used.

The invention relates also to animals, with the exception of human beings, comprising a transformed cell according to the invention.

In accordance with another aspect, the invention relates to a method of producing an antibody, or one of its functional fragments, according to the invention, characterised in that it comprises the following steps:

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

b) recovery of said antibodies, or a functional fragment thereof, thereby produced, from the culture medium or from said cultured cells.

The transformed cells according to the invention can be used in methods for the preparation of recombinant polypeptides according to the invention. The methods for the preparation of a polypeptide according to the invention in recombinant form, characterised in that they employ a vector and/or a cell transformed by a vector according to the invention, are themselves included in the present invention. Preferably, a cell transformed by a vector according to the invention is cultured under conditions enabling the expression of said polypeptide, and said recombinant peptide is recovered.

As has been stated, the cell host may be selected from prokaryotic or eukaryotic systems. In particular, it is possible to identify nucleotide sequences according to the invention which facilitate secretion in such a prokaryotic or eukaryotic system. A vector according to the invention carrying such a sequence can therefore be advantageously used in the production of recombinant proteins that are intended to be secreted. Indeed, purification of these recombinant proteins of interest will be facilitated by the fact that they are present in the supernatant of the cell culture rather than in the interior of the host cells.

It is also possible to prepare the polypeptides according to the invention by chemical synthesis. Such a preparation method is also included in the subject-matter of the invention. The person skilled in the art knows methods of chemical synthesis, for example techniques employing solid phases (see, especially, Steward *et al.*, 1984, Solid phase peptides synthesis, Pierce Chem. Company, Rockford, 111, 2nd Ed., (1984)) or techniques using partial solid phases, by means of condensation of fragments or by conventional synthesis in solution. The polypeptides obtained by chemical synthesis, which may contain corresponding non-natural amino acids, are also included in the invention.

The antibodies, or one of their derivative compounds or functional fragments, capable of being obtained by a method according to the invention are also included in the present invention.

According to yet another aspect, the present invention relates to an antibody as described hereinbefore, characterised in that it is, additionally, bispecific, that is to say capable of binding specifically to a human protein or human receptor other than CD151.

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 usefulness has been demonstrated both in the diagnostic field and in the therapeutic field by their ability to recruit new effector functions or to target a plurality of molecules on the surface of tumour cells. These 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, and preferably, by genetic engineering techniques which make it possible to force heterodimerisation and thereby facilitate the method of purifying the sought antibody (Merchand *et al.* 1998 Nature Biotech. 16: 677-681).

These bispecific antibodies can be constructed as whole IgGs, as bispecific Fab'2s, as Fab'PEGs or as diabodies or as bispecific scFvs but also as a tetravalent bispecific antibody in which two fixing sites are present for each antigen targeted (Park *et al.* 2000 Mol. Immunol. 37(18): 1123-30) or fragments thereof as described hereinbefore.

Besides an economic advantage due to the fact that production and administration of a bispecific antibody is less onerous than production of two specific antibodies, the use of such bispecific antibodies has the advantage of reducing the toxicity of the treatment. The use of a bispecific antibody makes it possible, in fact, to reduce the overall amount of circulating antibodies and, as a result, the possible toxicity.

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

Finally, the present invention is directed to the antibody, or one of its derivative compounds or functional fragments, as described hereinbefore, as a medicament.

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

According to yet another embodiment, the present invention relates also to a pharmaceutical composition as described hereinbefore which additionally comprises, as a combination product for simultaneous, separate or time-staggered use, at least one other antibody, a cytotoxic/cytostatic agent, a cell toxin or a radioelement.

"Simultaneous use" is understood as the administration of the two compounds of the composition according to the invention contained in one and the same pharmaceutical form.

"Separate use" is understood as the administration, at the same time, of the two compounds of the composition according to the invention contained in separate pharmaceutical forms.

"Time-staggered use" is understood as the successive administration of the two compounds of the composition according to the invention, each contained in a separate pharmaceutical form.

In general manner, the composition according to the invention considerably increases the efficacy of the cancer treatment. In other words, the therapeutic effect of the antibody according to the invention is potentiated in unexpected manner by the administration of a cytotoxic agent. Another major subsequent advantage produced by a composition according to the invention relates to the possibility of using lower effective doses of active ingredient, which makes it possible to avoid or reduce the risks of secondary effects appearing, especially the effect of the cytotoxic agent. Moreover, this composition according to the invention should make it possible to achieve the expected therapeutic effect more rapidly.

"Anti-cancer therapeutic agents" or "cytotoxic agents" should be understood as substances which, when administered to a patient, treats or prevents the development of the cancer in the patient. By way of non-limiting example of such agents there may be mentioned "alkylating" agents, antimetabolites, anti-tumour antibiotics, mitotic inhibitors, chromatin function inhibitors, anti-angiogenesis agents, anti-oestrogens, anti-androgens or immunomodulators.

Such agents are, for example, mentioned in the VIDAL, on the page devoted to oncology and haematology in the column "Cytotoxiques" (English: cytotoxic agents); such cytotoxic compounds mentioned by way of reference to that document are mentioned here as preferred cytotoxic agents.

"Alkylating agents" refer to any substance which is capable of covalently binding to or alkylating any molecule, preferably a nucleic acid (e.g.: DNA), within a cell. As examples of such alkylating agents there may be mentioned nitrogen mustards such as mechlorethamine, chlorambucil, melphalan hydrochloride, pipobroman, prednimustine disodium phosphate or estramustine; oxazophorines such as cyclophosphamide, altretamine, trofosfamide, sulfofosfamide or ifosfamide; aziridines

or ethylene-imines such as thiotepa, triethyleneamine or altetramine; nitrosoureas such as carmustine, streptozocin, fotemustine or lomustine; alkyl sulfonates such as busulfan, treosulfan or improsulfan; triazenes such as dacarbazine; and also platinum complexes such as cisplatin, oxaliplatin or carboplatin.

"Antimetabolites" refer to substances which block cell growth and/or cell metabolism by interfering with certain activities, generally DNA synthesis. By way of example of antimetabolites there may be mentioned methotrexate, 5-fluorouracil, floxuridine, 5-fluorodeoxyuridine, capecitabine, cytarabine, fludarabine, cytosine arabinoside, 6-mercaptopurine (6-MP), 6-thioguanine (6-TG), chlorodeoxyadenosine, 5-azacytidine, gemcitabine, cladribine, deoxycoformycin and pentostatin.

"Anti-tumour antibiotics" refer to compounds which can prevent or inhibit the synthesis of DNA, of RNA and/or of proteins. Examples of such anti-tumour antibiotics include doxorubicin, daunorubicin, idarubicin, valrubicin, mitoxantrone, dactinomycin, mithramycin, plicamycin, mitomycin C, bleomycin and procarbazine.

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

"Chromatin function inhibitors" or "topoisomerase inhibitors" refer to substances which inhibit the normal function of chromatin remodelling proteins such as topoisomerases I and II. Examples of such inhibitors include, for topoisomerase I, camptothecin and also its derivatives such as irinotecan or topotecan and, for topoisomerase II, etoposide, etiposide phosphate and teniposide.

"Anti-angiogenesis agents" refer to any drug, compound, substance or agent which inhibits the growth of blood vessels. Examples of anti-angiogenesis agents include, without any limitation, razoxin, marimastat, batimastat, prinomastat, tanomastat, ilomastat, CGS-27023A, halofuginone, COL-3, neovastat, BMS-275291, thalidomide, CDC 501, DMXAA, L-651582, squalamine, endostatin, SU5416, SU6668, interferon-alpha, EMD121974, interleukin-12, IM862, angiostatin and vitaxin.

"Anti-oestrogens" or "anti-oestrogen agents" refer to any substance which reduces, antagonises or inhibits the action of oestrogens. Examples of such agents are

tamoxifen, toremifene, raloxifene, droloxifene, iodoxyfene, anastrozole, letrozole and exemestane.

"Anti-androgens" or "anti-androgen agents" refer to any substance which reduces, antagonises or inhibits the action of an androgen. Examples of anti-androgens are flutamide, nilutamide, bicalutamide, spironolactone, cyproterone acetate, finasteride and cimitidine.

Immunomodulators are substances which stimulate the immune system. Examples of such immunomodulators include interferons, interleukins such as aldesleukin, OCT-43, denileukin diflitox or interleukin-2, tumour necrosis factors such as tasonermin, or other types of immunomodulators such as lentinan, sizofiran, roquinimex, pidotimod, pegademase, thymopentin, poly I:C, or levamisole in combination with 5-fluorouracil.

For further details, the person skilled in the art will be able to refer to the manual published by the French Association of Teachers of Therapeutic Chemistry entitled "Traité de chimie thérapeutique, Vol. 6, Médicaments antitumoraux et perspectives dans le traitement des cancers, ed. TEC & DOC, 2003".

In an especially preferred embodiment, said composition in the form of a combination product according to the invention is characterised in that said cytotoxic agent is chemically bound to said antibody for simultaneous use.

In an especially preferred embodiment, said composition according to the invention is characterised in that said cytotoxic/cytostatic agent is selected from spindle inhibitor or stabiliser agents, preferably vinorelbine and/or vinflunine and/or vincristine.

In order to facilitate binding between said cytotoxic agent and said antibody according to the invention, it will be possible, especially, to introduce spacer molecules between the two compounds to be bound, e.g. poly(alkylene)glycols such as polyethyleneglycol, or also amino acids, or, in another embodiment, to use active derivatives of said cytotoxic agents into which there will have been introduced functions capable of reacting with said antibody according to the invention. These binding techniques are will known to the person skilled in the art and will not be elaborated upon in the present description.

According to another aspect, the invention relates to a composition characterised in that one, at least, of said antibodies, or one of their derivative compounds or functional fragments, is conjugated with a cell toxin and/or a radioelement.

Preferably, said toxin or said radioelement is capable of preventing the growth or proliferation of the tumour cell, especially of totally inactivating said tumour cell.

Preference is also given to said toxin being an enterobacterial toxin, especially Pseudomonas exotoxin A.

The radioelements (or radioisotopes) employed in therapy, preferably conjugated with the antibody, are radioisotopes which emit gamma rays, preferably iodine^{131}, yttrium^{90}, gold^{199}, palladium^{100}, copper^{67}, bismuth^{217} and antimony^{211}. Radioisotopes which emit beta and alpha rays may also be used in therapy.

A toxin or radioelement conjugated with at least one antibody, or a functional fragment thereof, according to the invention is understood to refer to any means making it possible to bind said toxin or said radioelement to said at least one antibody, especially by covalent binding between the two compounds, with or without introduction of a linking molecule.

Among the agents allowing chemical (covalent), electrostatic or non-covalent linkage of all or some of the conjugate's elements there may be mentioned, very especially, benzoquinone, carbodiimide and, more especially, EDC (1-ethyl-3-[3-dimethylaminopropyl]-carbodiimide hydrochloride), dimaleimide, dithiobis-nitro-benzoic acid (DTNB), N-succinimidyl S-acetyl thioacetate (SATA), agents referred to as "bridging" agents having one or more groups, with one or more phenylazide groups, reacting with ultraviolet (UV) and very preferably N-[-4-(azidosalicylamino)butyl]-3'-(2'-pyridyldithio)propionamide (APDP), N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) and 6-hydrazino-nicotinamide (HYNIC).

Another form of binding, very especially for radioelements, can consist of using a bifunctional ion chelator.

Among those chelators there may be mentioned the chelators derived from EDTA (ethylenediaminetetraacetic acid) or DTPA (diethylenetriaminepentaacetic acid) that have been developed for binding metals, especially radioactive metals, and immunoglobulins. Accordingly, DTPA and its derivatives can be substituted with

different groups on the carbon chain so as to increase the stability and 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 has long been used very widely in medicine and biology either in its free form or in the form of a complex with a metal ion, has the noteworthy characteristic of forming stable chelates with metal ions and of being bound to proteins of therapeutic or diagnostic interest such as antibodies for the development of radioimmunoconjugates in cancer therapy (Meases *et al.*, (1984); Gansow *et al.* (1990)).

Also preferably, said at least one antibody forming said conjugate according to the invention is selected from its functional fragments, especially fragments lacking the Fc component such as scFv fragments.

The present invention additionally comprises use of the composition according to the invention in the preparation of a medicament intended for the prevention or treatment of cancer.

The present invention relates also to the use of an antibody, or one of its derivative compounds or functional fragments, preferably humanised, and/or of a composition according to the invention in the preparation of a medicament intended to inhibit the proliferation of tumour cells. In general manner, the present invention relates to the use of an antibody, or one of its derivative compounds or functional fragments, preferably humanised, and/or of a composition according to the invention in the preparation of a medicament intended for the prevention or treatment of cancer.

Among the cancers that may be prevented and/or treated, preference is given to prostate cancer, osteosarcomas, lung cancer, breast cancer, endometrial cancer, colon cancer, multiple myeloma or ovarian cancer, pancreatic cancer or any other cancer.

Preferably, said cancer is a cancer selected from prostate cancer, lung cancer, colon cancer, breast cancer and/or pancreatic cancer.

In accordance with yet another aspect, the present invention relates to the use of the antibody according to the invention in a, preferably *in vitro*, diagnostic method for diseases associated with a level of expression of CD151. More especially, the invention is directed to an *in vitro* diagnostic method for diseases having overexpression or

underexpression of the CD151 protein, starting from a biological sample in which the abnormal presence of the CD151 protein is suspected, said method consisting of placing said biological sample in contact with an antibody according to the invention, it being possible for said antibody, where appropriate, to be labelled.

Preferably, said diseases associated with the CD151 protein in said diagnostic method will be cancers.

Said antibody, or one of its functional fragments, can be in the form of an immunoconjugate or antibody labelled in order to obtain a detectable and/or quantifiable signal.

The antibodies labelled according to the invention or their functional fragments include, for example, antibodies referred to as immunoconjugates which can be conjugated, 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 with a molecule such as biotin, digoxigenin or 5-bromo-deoxyuridine. Fluorescent labels can also be conjugated with the antibodies, or their functional fragments, according to the invention and include, especially, fluorescein and its derivatives, fluorochrome, rhodamine and its derivatives, GFP (Green Fluorescent Protein), dansyl, umbelliferone etc.. In such conjugates, the antibodies of the invention or their functional fragments can be prepared by methods known to the person skilled in the art. They can be bound to the enzymes or fluorescent labels directly or by way of a spacer group or a linkage group such as a polyaldehyde, e.g. glutaraldehyde, ethylenediaminetetraacetic acid (EDTA) or diethylenetriaminepentaacetic acid (DPTA), or in the presence of binding agents such as those mentioned hereinbefore for the therapeutic conjugates. Conjugates comprising fluorescein-type labels can be prepared by reaction with an isothiocyanate.

Other conjugates can also include chemoluminescent labels such as luminol and dioxetanes, bioluminescent labels such as luciferase and luciferin, or also 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}, iodine^{131}. The methods known to the person skilled in the art that exist for binding radioisotopes to the antibodies, either directly or via a chelating agent such as EDTA or DTPA mentioned hereinbefore, can be used for the radioelements in diagnostics. There may also therefore be mentioned labelling with [I^{125}]Na by the chloramine T technique [Hunter W.M. and Greenwood F.C. (1962) Nature 194:495] or also with technetium^{99m} by the technique of Crockford *et al.* (US patent 4 424 200) or fixed via DTPA as described by Hnatowich (US patent 4 479 930).

The invention relates also to the use of an antibody according to the invention in the preparation of a medicament intended for the specific targeting of a biologically active compound at cells expressing or overexpressing the CD151 protein.

A biologically active compound is understood herein as referring to any compound capable of modifying, especially inhibiting, the activity of cells, especially their growth, their proliferation, or the transcription or translation of genes.

The invention relates also to an *in vivo* diagnostic reagent comprising an antibody according to the invention, or one of its functional fragments, preferably labelled, especially radiolabelled, and to its use in medical imaging, especially in the detection of cancer associated with the expression or overexpression of the CD151 protein by a cell.

The invention relates also to a composition in the form of a combination product or to an anti-CD151/toxin or radioelement conjugate, according to the invention, as a medicament.

Preferably, to said composition in the form of a combination product or to said conjugate according to the invention there will be added an excipient and/or a pharmaceutically acceptable carrier.

In the present description, a pharmaceutically acceptable carrier is understood as referring to a compound or combination of compounds included in a pharmaceutical composition which does not give rise to secondary reactions and which, for example, makes it possible to facilitate the administration of the active compound(s), to increase the life or efficacy thereof in the body, to increase the solubility thereof in solution or to improve its storage. Such pharmaceutically acceptable carriers are well-known and will

be adapted by the person skilled in the art as a function of the nature and mode of administration of the selected active compound(s).

Preferably, those compounds will be administered by a systemic route, especially the intravenous route, by the intramuscular, intradermic, intraperitoneal or subcutaneous route, or by the oral route. More preferably, the composition comprising the antibodies according to the invention will be administered on a plurality of occasions staggered over time.

Their optimal modes of administration, dosage regimens and galenic forms can be determined according to criteria generally taken into consideration in establishing a suitable treatment for a patient such as, for example, the age or bodyweight of the patient, the severity of his or her general condition, the tolerability of the treatment and the secondary effects established.

The invention relates accordingly to the use of an antibody, or one of its functional fragments, in the preparation of a medicament intended for specifically targeting a biologically active compound at cells expressing or overexpressing CD151.

Other characteristics and advantages of the invention will emerge in the remainder of the description with the Examples and Figures, for which the legends are given hereinbelow.

LEGENDS FOR FIGURES

Figure 1 shows the nucleotide and protein sequences of the CD151 protein, on which sequences there are shown the EC1 and EC2 loops.

Figure 2 is a diagram illustrating the structure of the tetraspanins, to which the CD151 protein belongs, and very especially the two extracellular loops EC1 and EC2.

Figure 3 shows the respective heavy chain and light chain sequences of antibody 203B6 according to the invention. The CDRs defined according to the IMGT system are shown underlined and in bold whereas the CDRs defined according to Kabat are shown in boxes.

Figure 4 shows the respective heavy chain and light chain sequences of antibody 205H8 according to the invention. The CDRs defined according to the IMGT system are

shown underlined and in bold whereas the CDRs defined according to Kabat are shown in boxes.

Figure 5 shows the respective heavy chain and light chain sequences of antibody 211F3 according to the invention. The CDRs defined according to the IMGT system are shown underlined and in bold whereas the CDRs defined according to Kabat are shown in boxes.

Figure 6 shows the respective heavy chain and light chain sequences of antibody 214B2 according to the invention. The CDRs defined according to the IMGT system are shown underlined and in bold whereas the CDRs defined according to Kabat are shown in boxes.

Figures 7A-7E illustrate the expression of the CD151 molecule in patients suffering from prostate cancer. Each letter corresponds to study of one patient and for each patient the upper panel corresponds to the normal tissue adjacent to the tumour and the lower panel corresponds to the tumour tissue.

Figure 8 illustrates the expression of the CD151 molecule in patients suffering from lung cancer. Each letter corresponds to study of one patient and for each patient the upper panel corresponds to the normal tissue adjacent to the tumour and the lower panel corresponds to the tumour tissue.

Figure 9 shows the analysis, by flow cytometry, of the recognition of CD151 by the murine antibody 203B6 on the surface of NIH 3T3-CD151, PC3 and A549 cells.

Figure 10 shows the analysis, by flow cytometry, of the recognition of CD151 by the murine antibody 205H8 on the surface of NIH 3T3-CD151, PC3 and A549 cells.

Figure 11 shows the analysis, by flow cytometry, of the recognition of CD151 by the murine antibody 211F3 on the surface of NIH 3T3-CD151, PC3 and A549 cells.

Figure 12 shows the analysis, by flow cytometry, of the recognition of CD151 by the murine antibody 214B2 on the surface of NIH 3T3-CD151, PC3 and A549 cells.

Figure 13 shows the results of evaluation of the specificity of antibodies 203B6, 205H8, 211F3 and 214B2 by Western blot.

Figure 14 illustrates the anti-tumour activity of anti-CD151 monoclonal antibodies in the PC3 prostate tumour xenograft model. The PC3 cells were grafted into Swiss Nude mice (n=6) by the subcutaneous route. Five days after grafting of the cells,

the mice receive, by the i.p. route, a challenge dose of 2 mg/mouse of the antibody under test followed by two administrations per week of a dose of 1 mg/mouse of that antibody. The tumour volume is determined by the formula $\pi/6$ x length x width x thickness.

Figure 15 shows the analysis of the antibodies 203B6 and 214B2 radiolabelled with iodine 125, with (A) Autoradiography after SDS-PAGE electrophoresis under reductive conditions and (B) Analysis by molecular-sieve chromatography.

Figure 16 illustrates the study of the fixing of the radiolabelled 203B6 antibody to PC3 cells: Determination of the affinity constant at equilibrium with (A) Saturation curve and (B) Scatchard plot.

Figure 17 illustrates the study of the fixing of the radiolabelled 214B2 antibody to PC3 cells: Determination of the affinity constant at equilibrium with (A) Saturation curve and (B) Scatchard plot.

Figure 18 shows the effect of the anti-CD151 antibodies on platelet functions, with (A) Platelet aggregation and (B) Platelet activation (release of serotonin).

Figure 19 shows the effect of anti-CD151 antibodies on the tumour growth, *in vivo*, of NCI-H441 cells.

Figure 20 illustrates the phenotype A) and differentiation markers B) of THP-1 cells cultured in the absence or presence of PMA. C) Effect of anti-CD151 antibodies (214B2 and 203B6) on the secretion of TNF by THP-1 cells cultured in the absence or presence of PMA. The antibody 9G4 is used as isotype control and LPS as positive activation control.

Figure 21 shows the effect of the antibodies 241B2 and 203B6 on antigen presentation. A) polyclonal proliferation control, B) antigen-specific proliferation.

EXAMPLES

Example 1: Study of expression of the CD151 molecule

The expression of the CD151 protein was researched by immunohistochemistry (IHC) in samples of human tissues obtained from patients suffering from prostate cancers or lung cancer. For these patients, slides of normal tissues adjacent to the tumour were available and were therefore included in order to calibrate the level of expression in the tumour tissues *versus* normal tissues.

For these experiments, commercially available slides of the "Tissue array" type are used. After deparaffinisation, antigen unmasking is performed at 30°C with the aid of an enzymatic solution containing pepsin (Labvision ref. AP-9007-005). This step is followed by a step of removal of endogenous peroxidases by incubation of the sections in a solution of hydrogen peroxide (Sigma) 0.3% in water. Saturation of the non-specific sites is then carried out with a solution of Ultra-V-Block (Labvision, ref. TA-125-UB) and labelling is carried out using a commercially available murine anti-CD151 antibody (Serotech, Ref. MCA 1856) used at a final concentration of 5 µg/ml. A murine IgG1 isotype control antibody (DakoCytomation, Ref. X0931) is used as a negative experimental control. Labelling visualisation is performed using the Envision Dual Link visualisation system (DakoCytomation, Ref. K4061) and the reference of the DAB peroxidase substrate is S3309 from DakoCytomation.

The results presented in Figure 7 show that a number of patients developing prostate tumours exhibit overexpression of the CD151 molecule. This overexpression may be very significant for 20% of the patients studied (patients A and C) or moderate (patients A and D). It is to be noted that, except at the level of the endothelial cells, the corresponding normal prostatic tissues do not express CD151 or express it only a little and that, where it is expressed, it seems to be limited to glandular type structures. Patient E exhibits an example of a tumour not expressing CD151.

In the case of the lung cancer (Figure 8), moderate (patient A) to marked (patient B) expression is observed in certain cells of normal pulmonary tissue. However, the tumour tissue exhibits a very high density of heavily labelled cells (patients A and B). Patient C exhibits an example of a tumour not expressing CD151.

Example 2: Generation and selection of the antibodies

BALB/c mice were immunised by the subcutaneous route using $20x10^6$ NIH 3T3 cells expressing human CD151 on their surface, those cells having been generated by transfection with the CD151 gene. The first immunisation was carried out in the presence of Freund's complete adjuvant, and the next 2 in the presence of Freund's incomplete adjuvant. Three days before fusion, a final booster injection of $10x10^6$ NIH 3T3-CD151 cells was carried out by the intraperitoneal route. Mouse spleen cells

were then fused to SP2/0-Ag14 myeloma cells in a ratio of 1/1 using conventional techniques described by Köhler and Milstein.

The antibodies secreted into the supernatants from the hybridomas resulting from the fusion were then screened for their ability to recognise the recombinant extracellular loop EC2 of CD151 by ELISA, and CD151 expressed on the surface of the human PC3 prostate cancer tumour line by flow cytometry.

For the ELISA, 96-well plates are sensitised for 1 night at +4°C with the recombinant extracellular loop EC2 at 5 µg/ml in PBS. After washing with PBS, the wells are saturated with 0.5% gelatin solution in PBS for 1 hour at 37°C and then washed again with PBS. The hybridoma culture supernatants are evaluated without dilution (incubation for 1 hour at 37°C). The antibodies fixed to the immobilised EC2 loop are detected by successive incubation with a peroxidase-conjugated goat anti-mouse IgG polyclonal antibody (Jackson/USA, dilution to 1/5000, 1 hour at 37°C) and then with a peroxidase substrate (TMB, Interchim/France, 10 minutes at ambient temperature). The reaction is stopped by addition of 1M sulfuric acid and the optical density (OD) is measured at 450 nm.

The flow cytometry analyses are carried out on 96-well plates. The undiluted hybridoma supernatants are added to 100000 PC3 cells previously introduced into the wells. After incubation for 20 minutes at +4°C followed by washing, an Alexa488-labelled goat anti-mouse IgG polyclonal antibody (Molecular Probes, dilution to 1/500) is added. The fluorescence intensity (MFI) is determined with the aid of a cytofluorimeter after further incubation for 20 minutes at +4°C.

At the end of that screening, the following 4 hybridomas were selected (selection criteria: OD > 0.5 for the ELISA and MFI > 50 for the flow cytometry): 203B6, 205H8, 211F3 and 214B2. The results obtained for those 4 hybridomas are presented in Table 4 below:

Table 4

Hybridoma	ELISA OD 450nm	Cytometry (PC3) MFI
203B6	1.130	975
205H8	0.649	595
211F3	0.959	936
214B2	0.684	1004

After cloning, a clone of each selected hybridoma was amplified. The isotypes of the antibodies produced were determined for each culture supernatant using a murine antibody isotyping kit (SBA clonotyping system, Southern Biotech), and then final characterisation was carried out by ELISA (extracellular loop EC2) and by flow cytometry on the murine line NIH 3T3 and the stable transfectant NIH 3T3-CD151, and then on human tumour lines of lung cancer A549, prostate cancer DU145 and pancreatic cancer BxPC3 under the conditions previously described. The antibody concentration of the supernatants was adjusted to 5 µg/ml for the ELISA and to 10 µg/ml for the flow cytometry analyses. The results obtained are presented in Table 5 below:

Table 5

Anti-CD151 antibody	Isotype	ELISA OD at 450nm	Flow cytometry MFI				
			NIH3T3	NIH3T3-CD151	A549	DU145	BxPC3
203B6 cl1A	IgG1 K	2.218	16	540	675	860	768
205H8 cl1A	IgG3 K	1.842	16	332	388	584	350
211F3 cl1A	IgG1 K	2.301	16	556	481	524	536
214B2 cl1A	IgG1 K	2.477	16	748	820	1332	849

Figures 9, 10, 11 and 12 show the recognition profiles for the NIH 3T3-CD151, PC3 and A549 cells, by flow cytometry, which are obtained for the antibodies 203B6, 205H8, 211F3 and 214B2. These profiles are comparable to those obtained with the

anti-CD151 antibody 50-6 (ATCC CRL-2696) and demonstrate the specificity of these antibodies for CD151.

The hybridomas were then deposited at the CNCM, Collection Nationale de Cultures de Microorganismes, Institut Pasteur, 25 Rue du Docteur Roux, 75724 PARIS Cedex 15.

Example 3: Specificity of the antibodies

The specificity of the 203B6, 205H8, 211F3 and 214B2 antibodies was evaluated by Western blot.

The EC2 loop of CD151 was cloned into the vector pET22b for expression in soluble form in the periplasm of Escherichia coli. The protein that is produced includes amino acids 130 to 221 of the human CD151 peptide sequence to which there is added a Poly-His tail at the C-terminal position in order to facilitate purification. The recombinant EC2 protein was purified by immobilised metal affinity chromatography (IMAC) on a Chelating Sepharose HP support (GE Healthcare).

Increasing amounts of the recombinant CD151 EC2 protein (1, 2, 4 and 8 µg) and of HT-29 cell lysate (10, 20 and 50 µg of total proteins) were placed on a 4-12% acrylamide gel (BioRad). After electrophoresis (non-reductive conditions), the proteins were transferred onto nitrocellulose membranes. The transfer membranes were then incubated with the purified 203B6, 205H8, 211F3 and 214B2 antibodies (0.5 µg/ml) and then with a rabbit anti-mouse Ig polyclonal antibody coupled to peroxidase (GE Healthcare) before visualisation by chemoluminescence.

The 203B6, 205H8, 211F3 and 214B2 antibodies recognise the complete CD151 protein and the recombinant EC2 loop by Western blot (Figure 13). The antibody 205H8 has less reactivity for the complete CD151 protein under the conditions of analysis. Compared to the signal observed for the antibodies 203B6, 211F3 and 214B2, the signal obtained for the antibody 205H8 is found to be weaker. This difference is not observed for recognition of the recombinant EC2 protein.

Example 4: Anti-tumour activity of anti-CD151 antibodies in the PC3 xenograft model

Material and methods

Having observed CD151 overexpression in tumour tissues of prostate cancer by means of immunohistochemistry on a human tissue array series, the evaluation of anti-CD151 antibodies on a PC3 prostate cancer cell xenograft was planned. The PC3 line is an androgen-independent prostate line obtained from ATCC and cultured in F12K medium (Invitrogen Corporation, Scotland, United Kingdom), 10% FCS (Invitrogen Corporation). The cells are divided two days before the graft so that they are in the exponential growth phase. Five million PC3 cells are subcutaneously grafted onto six-week-old male Swiss Nude mice. Five days after implantation, the tumour volumes are measured, the mice are randomised so as to form groups that are not statistically different, and the treatment is started by i.p. injection of a challenge dose of 2 mg of antibody per mouse. The animals are then treated twice a week with a dose of 1 mg of antibody per mouse and the tumours are measured twice a week. The animals of the control group are given an injection of PBS. The tumour volumes are calculated according to the formula $\pi/6$ x length x width x height. Statistical analysis is carried out on each measurement using a Mann-Whitney test.

Results

The results presented in Figure 14 show that the antibodies 203B6, 214B2 and 211F3 exhibit significant inhibition of the tumour growth *in vivo* of the PC3 cells. The antibody 205H8, whilst being an inhibitor, shows more modest anti-tumour activity in this model.

Example 5: Study of the fixing of anti-CD151 antibodies to PC3 cells

The anti-CD151 antibodies 203B6 and 214B2 were first labelled with iodine 125 by the method using chloramine T. After labelling, the antibodies were purified by molecular-sieve chromatography on a PD-10 column (GE Healthcare) in order to remove the free iodine 125. The radiochemical purity of the radiolabelled antibodies was determined, after purification, by silica thin-layer chromatography, analytical molecular-sieve chromatography on a Superdex 200 column (GE Healthcare) and by autoradiography after SDS-PAGE electrophoresis. Figure 15 shows that the heavy chains (~50 kDa) and light chains (~25 kDa) of the 2 antibodies are labelled in

equivalent manner (15A) and confirms the absence of free iodine 125 after purification (15B). The specific activity of the radiolabelled antibodies was determined using a gamma counter (Wallace Wizard 1480, Perkin Elmer).

The physico-chemical characteristics of the 203B6 and 214B2 antibodies radiolabelled with iodine 125 - [^{125}I]-203B6 and [^{125}I]-214B2 - are collated in Table 6 hereinbelow:

Table 6

Antibody	[^{125}I]-203B6	[^{125}I]-214B2
Labelling efficiency (%)	85.5	78.1
Specific activity (mCi/mg)	10.6	12.7
Radiochemical purity (%)	99.7	99.5
Atoms of iodine / antibody	0.73	0.87

The affinity of the 2 radiolabelled antibodies for their CD151 target on the cell surface was then measured for PC3 prostate cancer cells. The dissociation constant K_D of the antibodies was determined by the method of Scatchard. The PC3 cells (1×10^6 cells / 50 µl of PBS buffer containing 0.5 % BSA) were incubated in the presence of increasing concentrations of the radiolabelled antibodies between 6 ng/ml and 27 µg/ml, inclusive, for 1 hour at 4°C (final volume: 150 µl). After incubation, the total radioactivity associated with the cells (fixed radiolabelled antibody) was measured using a gamma counter. The non-specific fixing was determined, for each concentration tested, in the presence of an excess of non-radiolabelled antibody (x 100). The specific fixing is calculated by subtracting the non-specific radioactivity from the total radioactivity. The saturation curves are shown in Figures 16A and 17A for the antibodies [^{125}I]-203B6 and [^{125}I]-214B2, respectively. The Scatchard curves obtained (Figures 16B and 17B) after processing of the data using Prism software allow the dissociation constant K_D of the 2 antibodies (slope = - $1/K_D$) to be determined:

- 203B6 $K_D =$ 12.85 ± 0.99nM
- 214B2 $K_D =$ 6.38 ± 0.45nM

Example 6: Effect of the anti-CD151 antibodies on platelet functions

Aggregation is a basic function of platelets in the blood coagulation process, for example in response to a vascular lesion. This phenomenon is generally preceded by activation of the platelets, which involves exposure of certain proteins on their surface and secretion of the contents of their storage granules (alpha granules and dense granules). In view of the fact that CD151 is expressed on the platelets (Goschnick and Jackson, 2007, Mini-Rev. Med. Chem. 7: 1236-1247), the effect of anti-CD151 antibodies on platelet functions was evaluated *in vitro* using platelet aggregation and activation tests.

For this study, blood samples were taken from fasting donors using trisodium citrate as anticoagulation agent. Centrifuging at 100 g for 10 minutes at 20°C makes it possible to obtain platelet-rich plasma (PRP), whereas platelet-poor plasma (PPP) is obtained by centrifuging at 1500 g for 10 minutes at 20°C. Platelet aggregation was measured in the PRP of 10 donors, after adjustment to 300000 platelets/mm^3, according to Born's principle (modification of the light transmitted), with stirring at 500 rpm at 37°C. The measurement duration was 5 minutes for thrombin and ADP (positive controls) and 15 minutes for the antibodies tested. Platelet activation was measured in the PPP of 10 donors, by measuring the secretion of serotonin (5-hydroxytryptamine or 5-HT) using HPLC with fluorimetric detection. The effect of the antibodies on platelet functions was determined for a concentration of 10 µg/ml. Several control antibodies were used: PM6/248 (anti-CD41, integrin α_{IIb}) and 14A2.H1 (anti-CD151) as positive control antibodies, and 9G4 as isotype control (IgG1).

The platelet aggregation caused by the various compounds tested is assessed for each donor as a percentage of the aggregation caused by thrombin, which is taken as reference (100 %). Figure 18A shows the mean aggregation values obtained for each compound tested. ADP and the antibody PM6/248, agents which have a causative effect but which are less potent than thrombin, bring about comparable aggregation, 30.1 ± 10.5 % and 47.6 ± 12.7 %, respectively. The variations in the values obtained are routinely observed in haemostasis and result from the inter-individual variability. As expected, the 9G4 antibody does not cause platelet aggregation: this antibody allows a detection threshold (5nM) to be defined. The aggregation caused by the anti-CD151

antibodies 203B6 and 214B2, 7.3 ± 3.2 % and 4.9 ± 1.6 % respectively, is relatively low and close to the detection threshold. The aggregation caused by these 2 antibodies is less than that of the control antibody 14A2.H1.

Figure 18B shows the mean values for serotonin released by the platelets (in nM) in the presence of the various compounds tested. The results obtained for this test of platelet activation are comparable to those described above. The 9G4 antibody does not cause platelet activation whereas thrombin brings about a very marked release of serotonin (~430nM). The amounts of serotonin released are less in the presence of ADP, PM6/248 and 14A2.H1 (mean values of 72.6nM, 34.6nM and 21.6nM. respectively) but these compounds can be classed as platelet activators. The serotonin release caused by the anti-CD151 antibodies 203B6 and 214B2 is very low: in fact, the mean values determined for these 2 antibodies are 2.4 and 3.6 times lower, respectively, than the mean value for the control anti-CD151 antibody 14A2.H1.

In conclusion, the 2 tests confirm that the anti-integrin α_{IIb} control antibody PM6/248 and the anti-CD151 control antibody 14A2.H1 are capable of bringing about platelet aggregation *in vitro* and of causing platelet activation (Roberts *et al.*, 1995, Br. J. Haematol. 89: 853-860; Hornby *et al.*, 1991, Br. J. Haematol. 79: 277-285). In contrast to the 14A2.H1 antibody, the anti-CD151 antibodies 203B6 and 214B2 cause very low levels of platelet aggregation and activation which are not significant in human clinical terms.

Example 7: Study of the activity of anti-CD151 antibodies on the growth of NCI-H441 tumours *in vivo*

NCI-H441 cells obtained from the ATCC are cultured in RPMI 1640 medium to which 10 % FCS and 1 % L-glutamine have been added. The cells are divided two days before grafting so as to obtain cells that are in the exponential growth phase on the day of grafting. Ten million NCI-H441 cells are implanted at a subcutaneous location in athymic Nude mice. Five days after grafting, the tumours are measurable and the animals bearing tumours of comparable size are distributed into groups of 6. The mice are treated, by the i.p. route, with a challenge antibody dose of 2 mg/mouse and then twice a week with a dose of 1 mg of antibody per mouse. The tumour volume is

assessed twice a week and is calculated using the formula: $\pi/6$ X length X width X height. Specific analysis of the data is carried out at each measurement, using a Mann-Whitney test.

The results presented in Figure 19 demonstrate that the antibodies 203B6 and 214B2 are capable of significantly ($p<0.05$) inhibiting the growth, *in vivo*, of the NCI-H441 cells.

Example 8: Assessment of the activity of two anti-CD151 antibodies on macrophage function

Assessment of the expression of CD151 on cells of the blood shows that this molecule is expressed significantly on the lymphocytes and monocytes. In order to preclude any possible toxicity problems, the 214B2 and 203B6 antibodies were tested for a potential effect on macrophage activation. In view of the fact that secretion of TNFα is a marker of macrophage activation, the TNFα concentration of the supernatant from a culture of THP-1 cells, cultured in the presence or absence of the antibodies under test, was assessed by ELISA. The THP-1 cell line is a monocytic leukaemia line capable of differentiating into a macrophagic line in the presence of agents of the PMA or 1α,25-dihydroxyvitamin D3 type. This activation process is accompanied by very significant secretion of TNFα. For assessment of the antibodies, the THP-1 cells are seeded into 6-well plates in the presence or absence of PMA (250nM) for 72 hours. After that incubation period, some of the cells are recovered in order to check the differentiation in the presence of PMA by microscopic observation or by FACS analysis of the CD14/CD11b markers. The antibodies under test (241B2 and 203B6) are added to the other wells for an additional period of 24 hours. The 9G4 antibody is used under the same conditions as isotype control. LPS (1 μg/ml) is used as a positive experimental control. The culture supernatants are then withdrawn, centrifuged and stored at –80°C before being assayed for their TNFα content by means of an ELISA test.

The results shown in Figure 20A indicate that, in the absence of PMA, the THP-1 cells are non-adherent, refringent and round. In the presence of PMA, these cells become adherent, which is clearly indicative of differentiation of the monocytes into macrophages. The FACS analyses (Figure 20B) show that, in the absence of PMA, less

than 20 % of the THP-1 cells express CD11b or CD14. The cellular differentiation caused by the PMA is, as expected, associated with a significant increase in the cells expressing CD11b, which changes from 9 to 65 %. CD14 expression is also substantially increased, with the number of positive cells changing from 20 to 37 %. The validity of the assessment model employed having been demonstrated by this data, assessment of the anti-CD151 antibodies was carried out in this test. Figure 20C shows that no TNFα production is observed when the THP-1 cells are cultured in the absence of the differentiation inducer. In contrast, very significant production of TNFα (100 to 200 pg/ml) is observed. As expected, the LPS introduced as positive experimental control also brings about the secretion of a high level of cytokine, this being the case in the presence or absence of the differentiation inducer.

None of the anti-CD151 antibodies assessed causes secretion of TNFα whatever the state of cellular differentiation.

Example 9: Assessment of two anti-CD151 antibodies with respect to antigen presentation function

In view of the fact that the CD151 molecule is widely expressed on antigen-presenting cells, a study was carried out in order to check the potential impact, on antigen presentation function, of a therapy directed against CD151. The experiment performed consists of monitoring the specific lymphocyte proliferation of the TT antigen when the tetanus toxoid protein is introduced into PBMCs. In this arrangement, the protein should be processed by the antigen-presenting cells and exhibited by those same cells in association with the molecules of the MHC. This antigen presentation to CD4+ or CD8+ lymphocytes will cause proliferation of antigen-specific clones. Any impact on antigen presentation will therefore manifest itself in modulation of lymphocyte proliferation. For this experiment, PBMCs are isolated by centrifuging total blood on a Ficoll gradient. The cells, having been washed twice with PBS, are counted and re-suspended, at a concentration of 0.25 x 10^6 cells/ml, in RPMI 1640 medium to which 10 % FCS and 1 % glutamine have been added. 100 µl of the cell suspension are seeded into each well of a 96-well plate in the presence of the antigen and the antibodies under test. The 214B2 and 203B6 antibodies are assessed at a final concentration of

10 µg/ml. The 9G4 antibody is introduced at the same final concentration as experimental isotype control. PHA (2.5 µg/ml in well), a polyclonal activator of lymphocyte proliferation, is used as positive proliferation control. Tetanus toxoid (TT) was selected as test antigen and is used at a final concentration of 100 µg/ml. The plates are incubated at 37°C for 96 hours, at the end of which 0.25 µCi of ^{3}H-thymidine are added to each well for an additional period of 24 hours. After incubation, the cells are collected on filters and the radioactivity is assessed.

The results shown in Figure 21A indicate that, as expected, the PHA used as positive control causes very marked lymphocyte proliferation. The intensity of the signal agrees well with polyclonal activation of cell proliferation. The 9G4 included in the experiment as isotype control has no impact on cell proliferation in the absence or presence of PHA. Similarly, the two anti-CD151 antibodies tested do not, in either case, modify the proliferation. The TT antigen causes significant proliferation of part of the lymphocyte population (Figure 21B). There is no impact on this proliferation in the presence of 9G4 or of the two anti-CD151 antibodies tested, which indicates that, under the conditions of the experiment, antigen presentation is not affected by the compounds tested.

CLAIMS

1. Isolated antibody, or one of its derivative compounds or functional fragments, capable of binding to the CD151 protein and to inhibit the tumour growth *in vivo*, said antibody being selected in the group consisting of:

a) an antibody, or one of its derivative compounds or functional fragments, comprising:

i) a light chain comprising the 3 CDRs, as defined according to IMGT numbering system, CDR-L1 of sequence SEQ ID No.1, CDR-L2 of sequence SEQ ID No. 2 and CDR-L3 of sequence SEQ ID No. 3, respectively; and

ii) a heavy chain comprising the 3 CDRs, as defind according to IMGT numbering system, CDR-H1 of sequence SEQ ID No. 4, CDR-H2 of sequence SEQ ID No. 5 and CDR-H3 of sequence SEQ ID No. 6, respectively ;

b) an antibody, or one of its derivative compounds or functional fragments, comprising:

i) a light chain comprising the 3 CDRs, as defined according to IMGT numbering system, CDR-L1 of sequence SEQ ID No.17, CDR-L2 of sequence SEQ ID No. 18 and CDR-L3 of sequence SEQ ID No. 19, respectively; and

ii) a heavy chain comprising the 3 CDRs, as defind according to IMGT numbering system, CDR-H1 of sequence SEQ ID No. 20, CDR-H2 of sequence SEQ ID No. 21 and CDR-H3 of sequence SEQ ID No. 22, respectively;

c) an antibody, or one of its derivative compounds or functional fragments, comprising:

i) a light chain comprising the 3 CDRs, as defined according to IMGT numbering system, CDR-L1 of sequence SEQ ID No.33, CDR-L2 of sequence SEQ ID No. 2 and CDR-L3 of sequence SEQ ID No. 3, respectively; and

ii) a heavy chain comprising the 3 CDRs, as defind according to IMGT numbering system, CDR-H1 of sequence SEQ ID No. 4, CDR-H2 of sequence SEQ ID No. 34 and CDR-H3 of sequence SEQ ID No. 35, respectively; and

d) an antibody, or one of its derivative compounds or functional fragments, comprising:

i) a light chain comprising the 3 CDRs, as defined according to IMGT numbering system, CDR-L1 of sequence SEQ ID No.43, CDR-L2 of sequence SEQ ID No. 44 and CDR-L3 of sequence SEQ ID No. 45, respectively; and

ii) a heavy chain comprising the 3 CDRs, as defind according to IMGT numbering system, CDR-H1 of sequence SEQ ID No. 46, CDR-H2 of sequence SEQ ID No. 47 and CDR-H3 of sequence SEQ ID No. 48, respectively.

2. Antibody according to claim 1, being selected in the group consisting of:

a) an antibody, or one of its derivative compounds or functional fragments, comprising a light chain comprising the amino acid sequence SEQ ID No. 7 and a heavy chain comprising the amino acid sequence SEQ ID No. 8;

b) an antibody, or one of its derivative compounds or functional fragments, comprising a light chain comprising the amino acid sequence SEQ ID No. 23 and a heavy chain comprising the amino acid sequence SEQ ID No. 24;

c) an antibody, or one of its derivative compounds or functional fragments, comprising a light chain comprising the amino acid sequence SEQ ID No. 36 and a heavy chain comprising the amino acid sequence SEQ ID No. 37;

d) an antibody, or one of its derivative compounds or functional fragments, comprising a light chain comprising the amino acid sequence SEQ ID No. 49 and a heavy chain comprising the amino acid sequence SEQ ID No. 50.

3. Murine hybridoma capable of secreting an antibody according to any of the preceding claims, said hybridoma being selected in the group consisting of the hybridoma deposited at the CNCM, Institut Pasteur, Paris (France) on 22nd February 2008 under number I-3920; the hybridoma deposited at the CNCM, Institut Pasteur, Paris (France) on 22nd February 2008 under number I-3921; the hybridoma deposited at the CNCM, Institut Pasteur, Paris (France) on 21st February 2008 under number I-3918; and the hybridoma deposited at the CNCM, Institut Pasteur, Paris (France) on 21st February 2008 under number I-3919.

4. Isolated nucleic acid, characterised in that it is selected from the following nucleic acids:

a) a DNA or RNA nucleic acid coding for an antibody, or one of its derivative compounds or functional fragments, according to one of claims 1 and 2; and

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

5. Vector comprising a nucleic acid according to claim 4.

6. Cell host comprising a vector according to claim 5.

7. Transgenic animal, with the exception of a human being, comprising a cell according to claim 6.

8. Method of producing an antibody, or one of its derivative compounds or functional fragments, according to one of claims 1 and 2, characterised in that it comprises the following steps:

a) culture of a cell according to claim 6 in a suitable culture medium and under suitable culture conditions; and

b) recovery of said antibodies, or a functional fragment thereof, thereby produced, from the culture medium or from said cultured cells.

9. Antibody, or one of its derivative compounds or functional fragments, capable of being obtained by a method according to claim 8.

10. Composition comprising, as active ingredient, a compound consisting of an antibody, or one of its functional fragments, according to one of claims 1, 2 and 9 or
6 produced by the hybridoma according to claim 3.

11. Composition according to claim 10, characterised in that it additionally comprises, as a combination product for simultaneous, separate or time-staggered use, an antibody, a cytotoxic/cytostatic agent, a cell toxin or a radioelement.

12 12. Composition according to one of claims 10 and 11, as a medicament.

13. Use of an antibody, or one of its functional fragments, according to one of claims 1, 2 and 9 or produced by the hybridoma according to claim 3 and/or of a composition according to any one of claims 10 to 12, in the preparation of a medicament intended for the prevention or treatment of cancer.

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14. Use according to claim 13, characterised in that said cancer is a cancer selected from prostate cancer, lung cancer, colon cancer or pancreatic cancer.

15. *In vitro* diagnostic method for diseases having overexpression or underexpression of the CD151 protein, starting from a biological sample in which the abnormal presence
24 of the CD151 protein is suspected, characterised in that said biological sample is placed in contact with an antibody according to one of claims 1, 2 and 10 or produced by the hybridoma according to claim 3, it being possible for said antibody, where appropriate, to be labelled.

ASTRACT

The invention relates to novel antibodies that can bind specifically to the human CD151 protein, especially monoclonal antibodies of murine origin, chimeric antibodies, and humanised antibodies, and to the amino acid and nucleic acid sequences coding for said antibodies. The invention relates to the use of said antibodies as medicament for the prophylactic and/or therapeutic treatment of cancer and in methods or kits for diagnosing diseases related to the overexpression of the protein CD151. The invention further relates to products and/or compositions comprising such antibodies combined with anticancer antibodies and/or agents or conjugated with toxins and/or radioelements, and to use thereof for the prevention and/or treatment of certain cancers.

LISTAGE DE SEQUENCES

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