NOVEL ANTIBODY FOR THE DIAGNOSIS AND/OR PROGNOSIS OF CANCER

The present invention relates to the field of prognosis and/or diagnosis of a proliferative disease in a patient. More particularly, the invention relates to novel antibodies capable of binding specifically to the human cMet receptor, as well as the amino acid and nucleic acid sequences coding for these antibodies. The invention likewise comprises the use of said antibodies, and corresponding process, for detecting and diagnosing pathological hyperproliferative oncogenic disorders associated with expression of cMet. In certain embodiments, the disorders are oncogenic disorders associated with increased expression of cMet polypeptide relative to normal or any other pathology connected with the overexpression of cMet. The invention finally comprises products and/or compositions or kits comprising at least such antibodies for the prognosis or diagnostic of certain cancers.

Receptor tyrosine kinase (RTK) targeted agents such as trastuzumab, cetuximab, bevacizumab, imatinib and gefitinib inhibitors have illustrated the interest of targeting this class of proteins for treatment of selected cancers.

cMet, is the prototypic member of a sub-family of RTKs which also includes RON and SEA. The cMet RTK family is structurally different from other RTK families and is the only known high-affinity receptor for hepatocyte growth factor (HGF), also called scater factor (SF) [D.P. Bottaro et al., Science 1991, 251: 802-804; L. Naldini et al., Eur. Mol. Biol. Org. J. 1991, 10:2867-2878]. cMet and HGF are widely expressed in a variety of tissue and their expression is normally restricted to cells of epithelial and mesenchymal origin respectively [M.F. Di Renzo et al., Oncogene 1991, 6:1997-2003; E. Sonnenberg et al., J. Cell. Biol. 1993, 123:223-235]. They are both required for normal mammalian development and have been shown to be particularly important in cell migration, morphogenic differentiation, and organization of the three-dimensional tubular structures as well as growth and angiogenesis [F. Baldt et al., Nature 1995, 376:768-771; C. Schmidt et al., Nature. 1995:373:699-702; Tsarfaty et al., Science 1994, 263:98-101]. While the controlled regulation of cMet and HGF have been shown to be important in mammalian development, tissue maintenance and repair [Nagayama

T et al., Brain Res. 2004, 5;999(2):155-66; Tahara Y et al., J Pharmacol Exp Ther. 2003, 307(1):146-51], their dysregulation is implicated in the progression of cancers.

Aberrant signalling driven by inappropriate activation of cMet is one of the most frequent alteration observed in human cancers and plays a crucial role in tumorigenesis and metastasis [Birchmeier et al., Nat. Rev. Mol. Cell Biol. 2003, 4:915-925; L. Trusolino and Comoglio P. M., Nat Rev. Cancer. 2002, 2(4):289-300].

Inappropriate cMet activation can arise by ligand-dependent and independent mechanisms, which include overexpression of cMet, and/or paracrine or autocrine activation, or through gain in function mutation [J.G. Christensen, Burrows J. and Salgia R., Cancer Latters. 2005, 226:1-26]. However an oligomerization of cMet receptor, in presence or in absence of the ligand, is required to regulate the binding affinity and binding kinetics of the kinase toward ATP and tyrosine-containing peptide substrates [Hays JL et al., Biochemistry, 2004 Aug 17, 43:10570-8]. Activated cMet recruits signalling effectors to its multidocking site located in the cytoplasm domain, resulting in the activation of several key signalling pathways, including Ras-MAPK, PI3K, Src and Stat3 [Gao CF et al., Oncogene. 2000, 19(49):5582-9]. These pathways are essential for tumour cell proliferation, invasion and angiogenesis and for evading apoptosis [Furge KA et al., Trends Cell Biol. 2003 Mar, 13(3):122-30; Fan S et al., Oncogene. 2000 Apr 27, 19(18):2212-23]. In addition, a unique facet of the cMet signalling relative to other RTK is its reported interaction with focal adhesion complexes and non kinase binding partners such as $\alpha 6\beta 4$ integrins [Trusolino L et al., J Biol Chem. 1999, 274(10):6499-506], Plexin B1 or semaphorins [Giordano S et al., Nat Cell Biol. 2002, 4(9):720-4; Conrotto P et al., Blood. 2005, 105(11):4321-9; Conrotto P, Corso S, Gamberini S, Comoglio PM, Giordano S, Oncogene. 2004, 23:5131-7] which may further add to the complexity of regulation of cell function by this receptor. Finally recent data demonstrate that cMet could be involved in tumor resistance to gefitinib or erlotinib suggesting that combination of compound targeting both EGFR and cMet might be of significant interest [Engelman JA at al., Science, 2007, 316:1039-43].

In the past few years, many different strategies have been developed to attenuate cMet signalling in cancer cell lines. These strategies include i) neutralizing antibodies against cMet or HGF/SF [Cao B et al., Proc Natl Acad Sci U S A. 2001, 98(13):7443-8; Martens T et al., Clin Cancer Res. 2006, 12(20):6144-52] or the use of HGF/SF

antagonist NK4 to prevent ligand binding to cMet [Kuba K et al., Cancer Res., 2000, 60:6737-43], ii) small ATP binding site inhibitors to cMet that block kinase activity [Christensen JG et al., Cancer Res. 2003, 63:7345-55], iii) engineered SH2 domain polypeptide that interferes with access to the multidocking site and RNAi or ribozyme that reduce receptor or ligand expression. Most of these approaches display a selective inhibition of cMet resulting in tumor inhibition and showing that cMet could be of interest for therapeutic intervention in cancer.

The present invention aims to provide at least one reagent that can be used as a diagnostic or prognostic biomarker for detecting and/or monitoring oncogenic disorders especially those characterized by expression of cMet or those that are mediated by aberrant cMet expression.

Described herein are novel antibodies that meet this criteria.

Other features and advantages of the invention will be apparent from the detailed description and examples that follow.

In a first aspect, a subject of the invention is a binding protein, or a functional fragment or derivative thereof, that binds specifically to the ligand independent activated form of the cMet protein (cMet) preferably human cMet, with high affinity and can thus be useful in methods to diagnose pathological hyperproliferative oncogenic disorders mediated by ligand independent activated form of cMet expression.

A ligand-independent activation is related to a constitutive phosphorylation of cMet consecutive to i) either a dimerization of the receptor that occurrs in absence of HGF in a case of cMet overexpression (usually linked to an amplification of the *cMet* gene) or ii) to activating mutations within the intracellular domain of cMet or iii) both.

In a first aspect, the invention concerns a binding protein, or a functional fragment or derivative thereof, which on tumors i) specifically binds to the ligand independent activated form of cMet, but ii) does not bind to the non activated and/or ligand dependent activated form(s) of cMet.

By the expression « binding protein », it must be understood any peptidic chain having a specific or general affinity with another protein or molecule. Proteins are brought into contact and form a complex when binding is possible. The binding protein of the invention can preferably be, without limitation, an antibody, a fragment or derivative of an antibody, a protein or a peptide.

The expressions "functional fragment(s) and/or derivative(s)" will be defined in details later in the present specification.

By the expression "on tumor", it must be understand that the reported properties of the binding protein according to the invention are existing in the *in vivo* tumoral environment and not only on in vitro examples. More particularly, they have been demonstrated, as it will be apparent from the following examples, with *ex vivo* experiments representing an environment as closest as possible as the natural one or analysis on tissue microarray (TMA)-commercial of human tumor.

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

According to a embodiment of the invention, it is disclosed a binding protein, or a functional fragment or derivative thereof, as described before that does not block the binding of the ligand hepatocyte growth factor (HGF) to cMet.

More particularly, the binding protein, or a functional fragment or derivative thereof, of the invention interacts with the extra-cellular region of cMet between amino acid residues 1 and 950.

According to a first embodiment, the invention relates to a binding protein, or a functional fragment or derivative thereof, comprising at least one CDR chosen among the CDRs of sequences comprising at least SEQ ID No. 1, 2, 3, 4, 5, 6, 17, 18, 19, 20 or 21 or at least one CDR whose sequence has at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID No. 1, 2, 3, 4, 5, 6, 17, 18, 19, 20 or 21.

According to a second embodiment, the invention relates to a binding protein, or a functional fragment or derivative thereof, comprising at least one CDR chosen among the CDRs of sequences comprising at least SEQ ID No. 9, 10, 11, 12, 13, 14, 22, 23, 24, 25 or 26 or at least one CDR whose sequence has at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID No. 9, 10, 11, 12, 13, 14, 22, 23, 24, 25 or 26.

A "functional fragment" of an antibody means in particular an antibody

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

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

In a first embodiment, the binding protein, or a functional fragment or derivative thereof, of the invention comprises an antigen binding domain comprising at least one CDR selected from the group consisting of sequences SEQ ID Nos. 1-6.

In another embodiment, the binding protein, or a functional fragment or derivative thereof, of the invention comprises an antigen binding domain comprising at least one CDR selected from the group consisting of sequences SEQ ID Nos. 9-14.

Still in a preferred embodiment of the invention, said binding protein, or a functional fragment or derivative thereof, consists of an isolated antibody.

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

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

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

contrast with preparations of polyclonal antibodies which typically include various antibodies directed against various determinants, or epitopes, each monoclonal antibody is directed against a single epitope of the antigen.

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

More particularly, the antibody of the invention, or one of its functional fragments or derivatives, is characterized in that it comprises a light chain comprising at least one of the CDRs of the sequences SEQ ID Nos. 1, 2, 3, 17, 18 or at least one sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID Nos. 1, 2, 3, 17, 18.

Even more preferably, the antibody of the invention, or one of its functional fragments or derivatives, is characterized in that it comprises a light chain comprising the following three CDRs, respectively CDR-L1, CDR-L2 and CDR-L3, wherein:

- CDR-L1 comprises the sequence SEQ ID No. 1 or 17, or a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 1 or 17;

- CDR-L2 comprises the sequences SEQ ID No. 2 or 18, or a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 2 or 18; and

- CDR-L3 comprises the sequence SEQ ID No. 3, or a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 3.

The present invention thus describes an antibody, or a functional fragment or derivative thereof, which on tumors, i) specifically binds to the ligand independent activated form of cMet, but ii) does not bind to the non activated and/or ligand dependant activated form(s) of cMet, said antibody being characterized in that it comprises a light chain comprising the following three CDRs as defined according to IMGT, respectively CDR-L1, CDR-L2 and CDR-L3, wherein CDR-L1 comprises the sequence SEQ ID No. 1, CDR-L2 comprises the sequence SEQ ID No. 2 and CDR-L3 comprises the sequence SEQ ID No. 3.

According to another particular embodiment, the antibody of the invention, or one of its functional fragments or derivatives, is characterized in that it comprises a light

chain comprising the following three CDRs as defined according to Kabat, respectively CDR-L1, CDR-L2 and CDR-L3, wherein CDR-L1 comprises the sequence SEQ ID No. 17, CDR-L2 comprises the sequence SEQ ID No. 18 and CDR-L3 comprises the sequence SEQ ID No. 3.

According to another embodiment, the antibody of the invention, or one of its functional fragments or derivatives, is characterized in that it comprises a heavy chain comprising at least one of the CDRs of the sequences SEQ ID Nos. 4, 5, 6, 19, 20 or 21 or at least one sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID Nos. 4, 5, 6, 19, 20 or 21.

In a preferred manner, the antibody of the invention, or one of its functional fragments or derivatives, is characterized in that it comprises a heavy chain comprising the following three CDRs, respectively CDR-H1, CDR-H2 and CDR-H3, wherein:

- CDR-H1 comprises the sequence SEQ ID No. 4 or 19, or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 4 or 19;

- CDR-H2 comprises the sequences SEQ ID No. 5 or 20, or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 5 or 20; and

- CDR-H3 comprises the sequence SEQ ID No. 6 or 21, or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 6 or 21.

The present invention thus describes an antibody, or a functional fragment or derivative thereof, which on tumors i) specifically binds to the ligand independent activated form of cMet, but ii) does not bind to the non activated and/or ligand dependant activated form(s) of cMet, said antibody being characterized in that it comprises a heavy chain comprising the following three CDRs as defined according to IMGT, respectively CDR-H1, CDR-H2 and CDR-H3, wherein CDR-H1 comprises the sequence SEQ ID No. 4, CDR-H2 comprises the sequence SEQ ID No. 5 and CDR-H3 comprises the sequence SEQ ID No. 6.

According to another particular embodiment, the antibody of the invention, or one of its functional fragments or derivatives, is characterized in that it comprises a heavy chain comprising the following three CDRs as defined according to Kabat,

respectively CDR-H1, CDR-H2 and CDR-H3, wherein CDR-H1 comprises the sequence SEQ ID NO. 19, CDR-H2 comprises the sequence SEQ ID No. 20 and CDR-H3 comprises the sequence SEQ ID No. 21.

More particularly, the antibody of the invention, or one of its functional fragments or derivatives, is characterized in that it comprises a light chain comprising at least one of the CDRs of the sequences SEQ ID Nos. 9, 10, 11, 22, 23 or at least one sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID Nos. 9, 10, 11, 22, 23.

Even more preferably, the antibody of the invention, or one of its functional fragments or derivatives, is characterized in that it comprises a light chain comprising the following three CDRs, respectively CDR-L1, CDR-L2 and CDR-L3, wherein:

- CDR-L1 comprises the sequence SEQ ID No. 9 or 22, or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 9 or 22;
- CDR-L2 comprises the sequences SEQ ID No. 10 or 23, or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 10 or 23; and
- CDR-L3 comprises the sequence SEQ ID No. 11, or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 11.

The present invention thus describes an antibody, or a functional fragment or derivative thereof, which on tumors i) specifically binds to the ligand independent activated form of cMet, but ii) does not bind to the non activated and/or ligand dependant activated form(s) of cMet, said antibody being characterized in that it comprises a light chain comprising the following three CDRs as defined according to IMGT, respectively CDR-L1, CDR-L2 and CDR-L3, wherein CDR-L1 comprises the sequence SEQ ID No. 9, CDR-L2 comprises the sequence SEQ ID No. 10 and CDR-L3 comprises the sequence SEQ ID No. 11.

According to another particular embodiment, the antibody of the invention, or one of its functional fragments or derivatives, is characterized in that it comprises a light chain comprising the following three CDRs as defined according to Kabat, respectively CDR-L1, CDR-L2 and CDR-L3, wherein CDR-L1 comprises the sequence SEQ ID No.

22, CDR-L2 comprises the sequence SEQ ID No. 23 and CDR-L3 comprises the sequence SEQ ID No. 11.

According to another embodiment, the antibody of the invention, or one of its functional fragments or derivatives, is characterized in that it comprises a heavy chain comprising at least one of the CDRs of the sequences SEQ ID Nos. 12, 13, 14, 24, 25, 26 or at least one sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequences SEQ ID Nos. 12, 13, 14, 24, 25, 26.

In a preferred manner, the antibody of the invention, or one of its functional fragments or derivatives, is characterized in that it comprises a heavy chain comprising the following three CDRs, respectively CDR-H1, CDR-H2 and CDR-H3, wherein:

- CDR-H1 comprises the sequence SEQ ID No. 12 or 24, or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 12 or 24;

- CDR-H2 comprises the sequences SEQ ID No. 13 or 25, or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 13 or 25; and

- CDR-H3 comprises the sequence SEQ ID No. 14 or 26, or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 14 or 26.

The present invention thus describes an antibody, or a functional fragment or derivative thereof, which on tumors i) specifically binds to the ligand independent activated form of cMet, but ii) does not bind to the non activated and/or ligand dependant activated form(s) of cMet, said antibody being characterized in that it comprises a heavy chain comprising the following three CDRs as defined according to IMGT, respectively CDR-H1, CDR-H2 and CDR-H3, wherein CDR-H1 comprises the sequence SEQ ID No. 12, CDR-H2 comprises the sequence SEQ ID No. 13 and CDR-H3 comprises the sequence SEQ ID No. 14.

According to another particular embodiment, the antibody of the invention, or one of its functional fragments or derivatives, is characterized in that it comprises a heavy chain comprising the following three CDRs as defined according to Kabat, respectively CDR-H1, CDR-H2 and CDR-H3, wherein CDR-H1 comprises the sequence SEQ ID No. 24, CDR-H2 comprises the sequence SEQ ID No. 25 and CDR-

H3 comprises the sequence SEQ ID No. 26.

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

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

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

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

The IMGT unique numbering has been defined to compare the variable domains whatever the antigen receptor, the chain type, or the species [Lefranc M.-P., Immunology Today 18, 509 (1997) / Lefranc M.-P., The Immunologist, 7, 132-136 (1999) / Lefranc, M.-P., Pommié, C., Ruiz, M., Giudicelli, V., Foulquier, E., Truong, L., Thouvenin-Contet, V. and Lefranc, Dev. Comp. Immunol., 27, 55-77 (2003)]. In the IMGT unique numbering, the conserved amino acids always have the same position, for instance cystein 23 (1st-CYS), tryptophan 41 (CONSERVED-TRP), hydrophobic amino acid 89, cystein 104 (2nd-CYS), phenylalanine or tryptophan 118 (J-PHE or J-TRP). The IMGT unique numbering provides a standardized delimitation of the framework regions (FR1-IMGT: positions 1 to 26, FR2-IMGT: 39 to 55, FR3-IMGT: 66 to 104 and FR4-IMGT: 118 to 128) and of the complementarity determining regions: CDR1-IMGT: 27 to 38, CDR2-IMGT: 56 to 65 and CDR3-IMGT: 105 to 117. As gaps

represent unoccupied positions, the CDR-IMGT lengths (shown between brackets and separated by dots, e.g. [8.8.13]) become crucial information. The IMGT unique numbering is used in 2D graphical representations, designated as IMGT Colliers de Perles [Ruiz, M. and Lefranc, M.-P., Immunogenetics, 53, 857-883 (2002) / Kaas, Q. and Lefranc, M.-P., Current Bioinformatics, 2, 21-30 (2007)], and in 3D structures in IMGT/3Dstructure-DB [Kaas, Q., Ruiz, M. and Lefranc, M.-P., T cell receptor and MHC structural data. Nucl. Acids. Res., 32, D208-D210 (2004)].

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

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

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

The percentage identity between two nucleic acid or amino acid sequences is

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

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

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

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

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

possible under the same conditions.

Table 1

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

It is known by those skilled in the art that in the current state of the art the greatest variability (length and composition) between the six CDRs is found at the three heavy-chain CDRs and, more particularly, at CDR-H3 of this heavy chain.

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

Another embodiment of the invention discloses an antibody, or one of its

functional fragments or derivatives, comprising a light chain comprising the following three CDRs according to IMGT:

- CDR-L1 of the sequence SEQ ID No. 1 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 1;

- CDR-L2 of the sequence SEQ ID No. 2 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 2; and

- CDR-L3 of the sequence SEQ ID No. 3 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 3, and

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

- CDR-H1 of the sequence SEQ ID No. 4 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 4;

- CDR-H2 of the sequence SEQ ID No. 5 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 5; and

- CDR-H3 of the sequence SEQ ID No. 6 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 6.

In a preferred embodiment, the antibody, or a functional fragment or derivative thereof, of the invention which on tumors i) specifically binds to the ligand independent activated form of cMet, but ii) does not bind to the non activated and/or ligand dependant activated form(s) of cMet, comprises a) a light chain comprising the following three CDRs as defined according to IMGT, respectively CDR-L1, CDR-L2 and CDR-L3, wherein CDR-L1 comprises the sequence SEQ ID No. 1, CDR-L2 comprises the sequence SEQ ID No. 2 and CDR-L3 comprises the sequence SEQ ID No. 3 and b) a heavy chain comprising the following three CDRs as defined according to IMGT, respectively CDR-H1, CDR-H2 and CDR-H3, wherein CDR-H1 comprises the sequence SEQ ID No. 4, CDR-H2 comprises the sequence SEQ ID No. 5 and CDR-H3 comprises the sequence SEQ ID No. 6.

Still another embodiment of the invention discloses an antibody, or one of its functional fragments or derivatives, comprising a light chain comprising the following three CDRs according to IMGT:

- CDR-L1 of the sequence SEQ ID No. 9 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 9;

- CDR-L2 of the sequence SEQ ID No. 10 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 10; and

- CDR-L3 of the sequence SEQ ID No. 11 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 11, and

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

- CDR-H1 of the sequence SEQ ID No. 12 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 12;

- CDR-H2 of the sequence SEQ ID No. 13 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 13; and

- CDR-H3 of the sequence SEQ ID No. 14 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 14.

In a preferred embodiment, the antibody, or a functional fragment or derivative thereof, of the invention which on tumors i) specifically binds to the ligand independent activated form of cMet, but ii) does not bind to the non activated and/or ligand dependant activated form(s) of cMet, comprises a) a light chain comprising the following three CDRs as defined according to IMGT, respectively CDR-L1, CDR-L2 and CDR-L3, wherein CDR-L1 comprises the sequence SEQ ID No. 9, CDR-L2 comprises the sequence SEQ ID No. 10 and CDR-L3 comprises the sequence SEQ ID No. 11 and b) a heavy chain comprising the following three CDRs as defined according to IMGT, respectively CDR-H1, CDR-H2 and CDR-H3, wherein CDR-H1 comprises the sequence SEQ ID No. 12, CDR-H2 comprises the sequence SEQ ID No. 13 and

CDR-H3 comprises the sequence SEQ ID No. 14.

Still another embodiment of the invention discloses an antibody, or one of its functional fragments or derivatives, comprising a light chain comprising the following three CDRs according to Kabat:

- CDR-L1 of the sequence SEQ ID No. 17 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 17;

- CDR-L2 of the sequence SEQ ID No. 18 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 18; and

- CDR-L3 of the sequence SEQ ID No. 3 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 3, and

a heavy chain comprising the following three CDRs according to Kabat:

- CDR-H1 of the sequence SEQ ID No. 19 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 19;

- CDR-H2 of the sequence SEQ ID No. 20 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 20; and

- CDR-H3 of the sequence SEQ ID No. 21 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 21.

Still another embodiment of the invention discloses an antibody, or one of its functional fragments or derivatives, comprising a light chain comprising the following three CDRs according to Kabat:

- CDR-L1 of the sequence SEQ ID No. 22 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 22;

- CDR-L2 of the sequence SEQ ID No. 23 or of a sequence with at least 80% identity after optimal alignment with sequence SEQ ID No. 23; and

- CDR-L3 of the sequence SEQ ID No. 11 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence

SEQ ID No. 11, and

a heavy chain comprising the following three CDRs according to Kabat:

- CDR-H1 of the sequence SEQ ID No. 24 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 24;

- CDR-H2 of the sequence SEQ ID No. 25 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 25; and

- CDR-H3 of the sequence SEQ ID No. 26 or of a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 26.

According to still another embodiment, the antibody of the invention, or a functional fragment or derivative thereof, is characterized in that it comprises a light chain variable domain of sequence comprising the amino acid sequence SEQ ID No. 7 or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 7; and/or a heavy chain variable domain of sequence comprising the amino acid sequence SEQ ID No. 8 or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 8.

The invention thus describes an antibody, or a functional fragment or derivative thereof, which on tumors i) specifically binds to the ligand independent activated form of cMet, but ii) does not bind to the non activated and/or ligand dependant activated form(s) of cMet, said antibody comprising a light chain variable domain of sequence comprising the amino acid sequence SEQ ID No. 7 and a heavy chain variable domain of sequence comprising the amino acid sequence SEQ ID No. 8.

According to still another embodiment, the antibody of the invention, or a functional fragment or derivative thereof, is characterized in that it comprises a light chain variable domain of sequence comprising the amino acid sequence SEQ ID No. 15 or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with sequence SEQ ID No. 15; and/or a heavy chain variable domain of sequence comprising the amino acid sequence SEQ ID No. 16 or a sequence with at least 80%, preferably 85%, 90%, 95% and 98% identity after optimal alignment with

sequence SEQ ID No. 16.

The invention thus describes an antibody, or a functional fragment or derivative thereof, which on tumors i) specifically binds to the ligand independent activated form of cMet, but ii) does not bind to the non activated and/or ligand dependant activated form(s) of cMet, said antibody comprising a light chain variable domain of sequence comprising the amino acid sequence SEQ ID No. 15 and a heavy chain variable domain of sequence comprising the amino acid sequence SEQ ID No. 16.

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

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

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

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

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

The origin of such protein scaffolds can be, but is not limited to, the structures selected among: fibronectin and preferentially fibronectin type III domain 10, lipocalin, anticalin (Skerra A., J. Biotechnol., 2001, 74(4):257–75), protein Z arising from domain

B of protein A of *Staphylococcus aureus*, thioredoxin A or proteins with a repeated motif such as the "ankyrin repeat" (Kohl *et al.*, PNAS, 2003, vol. 100, No. 4, 1700-1705), the "armadillo repeat", the "leucine-rich repeat" and the "tetratricopeptide repeat".

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

An example, in no way limiting, of such hybrid constructions, is the insertion of the CDR-H1 (heavy chain) of an anti-CD4 antibody, namely 13B8.2, in one of the loops in the PIN, the new binding protein thus obtained preserving the same binding properties as the original antibody (Bes *et al.*, Biochem. Biophys. Res. Commun., 2006, 343(1), 334-344). On a purely illustrative basis, grafting the CDR-H3 (heavy chain) of an anti-lysozyme VHH antibody on one of the loops of neocarzinostatin (Nicaise *et al.*, Protein Science, 2004, 13(7):1882-1891) can also be mentioned.

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

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

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

protein inhibiters of neuronal NO synthase (PIN).

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

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

Such functional fragments of the antibody according to the invention consist, for example, of the fragments Fv, scFv (sc=simple chain), Fab, $F(ab')_2$, Fab', scFv-Fc or diabodies, or any fragment whose half-life has been increased by chemical modification, such as the addition of polyalkylene glycol such as polyethylene glycol (PEGylation) (PEGylated fragments are referred to as Fv-PEG, scFv-PEG, Fab-PEG, $F(ab')_2$-PEG and Fab'-PEG), or by incorporation in a liposome, microspheres or PLGA, said fragments possessing at least one of the characteristic CDRs of the invention which is notably capable of exerting in a general manner activity, even partial, of the antibody from which it arises.

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

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

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

those sold by Applied BioSystems, etc.

For more clarity, table 2a below summarizes the various amino acid sequences corresponding to the antibody of the invention according to IMGT whereas table 2b summarizes the various amino acid sequences corresponding to the antibody of the invention according to Kabat.

Table 2a (wherein Mu. = murine)

Antibody	CDR numbering	Heavy chain	Light chain	SEQ ID NO.
227D3	IMGT		CDR-L1	1
			CDR-L2	2
			CDR-L3	3
		CDR-H1		4
		CDR-H2		5
		CDR-H3		6
			Mu. variable domain	7
		Mu. variable domain		8
205A5	IMGT		CDR-L1	9
			CDR-L2	10
			CDR-L3	11
		CDR-H1		12
		CDR-H2		13
		CDR-H3		14
			Mu. variable domain	15
		Mu. variable domain		16

Table 2b (wherein Mu. = murine)

Antibody	CDR numbering	Heavy chain	Light chain	SEQ ID NO.
227D3	Kabat		CDR-L1	17
			CDR-L2	18
			CDR-L3	3
		CDR-H1		19
		CDR-H2		20
		CDR-H3		21
205A5	Kabat		CDR-L1	22
			CDR-L2	23
			CDR-L3	11
		CDR-H1		24
		CDR-H2		25
		CDR-H3		26

According to another aspect, the invention relates to a murine hybridoma capable of secreting a monoclonal antibody according to the invention, notably the hybridoma of murine origin deposited at the CNCM, Institut Pasteur, Paris, France, on November 18, 2009, under the number I-4247. Said hybridoma was obtained by the fusion of Balb/C immunized mice splenocytes and cells of the myeloma Sp 2/O-Ag 14 lines.

The monoclonal antibody, here referred to as 227D3, or its derived compounds or functional fragments, characterized in that said antibody is secreted by the hybridoma deposited at the CNCM on November 18, 2009, under number I-4247 obviously forms part of the present invention.

The invention thus also encompasses a monoclonal antibody derived from hybridoma I-4247 or a subclone thereof which on tumors i) specifically binds to the ligand independent activated form of cMet, but ii) does not bind to the non activated and/or ligand dependant activated form(s) of cMet.

According to another aspect, the invention relates to a murine hybridoma capable of secreting a monoclonal antibody according to the invention, notably the hybridoma of murine origin deposited at the CNCM, Institut Pasteur, Paris, France, on

November 18, 2009, under the number I-4246. Said hybridoma was obtained by the fusion of Balb/C immunized mice splenocytes and cells of the myeloma Sp 2/O-Ag 14 lines.

The monoclonal antibody, here referred to as 205A5, or its derived compounds or functional fragments, characterized in that said antibody is secreted by the hybridoma deposited at the CNCM on November 18, 2009, under number I-4246 obviously forms part of the present invention.

The invention thus also encompasses a monoclonal antibody derived from hybridoma I-4246 or a subclone thereof which on tumors i) specifically binds to the ligand independent activated form of cMet, but ii) does not bind to the non activated and/or ligand dependant activated form(s) of cMet.

In another embodiment, the invention deals with an isolated nucleic acid, characterized in that it is chosen from the following nucleic acids:

a) a nucleic acid, DNA or RNA, coding for a binding protein as above described;

b) a nucleic acid, DNA or RNA, coding for an antibody as above described;

c) a nucleic acid comprising a DNA sequence comprising the sequences SEQ ID Nos. 27 to 32 or 35 to 40;

d) a nucleic acid comprising a DNA sequence comprising the sequences SEQ ID Nos. 33, 34, 41 or 42;

e) the corresponding RNA nucleic acids of the nucleic acids as defined in c) or d); and

f) the complementary nucleic acids of the nucleic acids as defined in a), b), c) and d).

Table 3 below summarizes the various nucleotide sequences concerning the antibody of the invention. All the CDRs are herein defined according to IMGT (Definition of the CDRs according to Kabat are not represented in table 3 but, for the man skilled in the art, it will be obvious to define them taking into consideration the sequences of table 2b).

Table 3

Antibody	CDR numbering	Heavy chain	Light chain	SEQ ID NO.
227D3	IMGT		CDR-L1	27
			CDR-L2	28
			CDR-L3	29
		CDR-H1		30
		CDR-H2		31
		CDR-H3		32
			Mu. variable domain	33
		Mu. variable domain		34
205A5	IMGT		CDR-L1	35
			CDR-L2	36
			CDR-L3	37
		CDR-H1		38
		CDR-H2		39
		CDR-H3		40
			Mu. variable domain	41
		Mu. variable domain		42

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

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

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

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

DNA-DNA or DNA-RNA hybridization is carried out in two steps: (1) prehybridization at 42°C for three hours in phosphate buffer (20 mM, pH 7.5) containing 5X SSC (1X SSC corresponds to a solution of 0.15 M NaCl + 0.015 M sodium citrate), 50% formamide, 7% sodium dodecyl sulfate (SDS), 10X Denhardt's, 5% dextran sulfate and 1% salmon sperm DNA; (2) primary hybridization for 20 hours at a temperature depending on the length of the probe (i.e.: 42°C for a probe >100 nucleotides in length) followed by two 20-minute washings at 20°C in 2X SSC + 2% SDS, one 20–minute washing at 20°C in 0.1X SSC + 0.1% SDS. The last washing is carried out in 0.1X SSC + 0.1% SDS for 30 minutes at 60°C for a probe >100 nucleotides in length. The highly stringent hybridization conditions described above for a polynucleotide of defined size can be adapted by a person skilled in the art for longer or shorter oligonucleotides, according to the procedures described in Sambrook, *et al.* (Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory; 3rd edition, 2001).

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

The invention notably targets cloning and/or expression vectors that contain

such a nucleotide sequence.

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

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

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

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

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

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

Another aspect of the invention relates to a method for the production of an antibody capable of binding specifically to the ligand independent activated form of cMet protein, according to the invention, or one of its functional fragments, characterized in that said method comprises the following steps:

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

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

The transformed cells according to the invention are of use in methods for the

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

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

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

The invention also comprises the use of a binding protein or a monoclonal antibody according to the invention, or a functional fragment or derivative thereof, for the identification of the ligand independent activated form of cMet.

In another embodiment, the invention concerns a process for differentiating between the ligand independent activated form of cMet and the others forms of cMet, including non activated or ligand dependent activated forms of cMet, in a sample, which process comprises the steps of:

a) contacting said sample with a binding or an antibody according to the

invention, or a functional fragment or derivative thereof, and

b) detecting the binding of said binding protein or antibody with the sample.

The use of the binding protein, and more particularly the antibody, of the invention as biomarker is also disclosed. The methods may be used for detecting or diagnosing various hyperproliferative oncogenic disorders associated with expression of ligand-independent activated form of cMet, exemplified by, but not limited to, osteosarcomas, lung cancer, breast cancer, endometrial cancer, glioblastoma, colon, cancer, gastric cancer, renal cancer, hepathocarcinomas or any other cancer associated with expression of ligand independent activated form of cMet. As would be recognized by one of ordinary skill in this art, the level of binding protein and/or antibody expression associated with a particular disorder will vary depending on the nature and/or the severity of the pre-existing condition.

In another aspect, the present inventions is directed to an *in vivo* method for detecting or diagnosing or staging in a patient hyperproliferative oncogenic disorders associated with expression of ligand independent activated form of cMet, particularly said hyperproliferative oncogenic disorders as cited above, said method comprising the steps of:

a) administration of the binding protein and/or antibodies of the present invention, preferably labeled, to the patient in need thereof; and

b) detecting, preferably by imaging, the binding of said binding protein or antibody with ligand independent activated form of cMet expressed in the patient, preferably by the patient organ wherein the presence of tumoral cells or tumor is suspected or known (particularly for the staging).

Administration of the binding protein and/or antibodies of the present invention in any of the conventional ways known to one skilled in the art (e.g., topical, parenteral, intramuscular, etc.), will provide an extremely useful method of detecting dysplastic cells in a sample as well as allowing a clinician to monitor the therapeutic regiment of a patient undergoing treatment for a hyperproliferative disorder associated with or mediated by expression of ligand independent activated form of cMet.

In another embodiment, the invention relates to a pharmaceutical composition for *in vivo* imaging of an oncogenic disorder associated with expression of ligand independent activated form of cMet, comprising the above binding protein and/or

antibody or fragment thereof which is labeled and which binds ligand independent activated form of cMet *in vivo*; and a pharmaceutically acceptable carrier.

The binding protein and the antibody of the invention, or a functional fragment or derivative thereof, will be used in various medical or research purposes, including the detection, diagnosis, and staging of various pathologies associated with expression of ligand independent activated form of cMet.

Stage determination has potential prognostic value and provides criteria for designing optimal therapy. Simpson et al., J. Clin. Oncology 18:2059 (2000). Generally, pathological staging of breast cancer for example, is preferable to clinical staging because the former gives a more accurate prognosis. However, clinical staging would be preferred if it were as accurate as pathological staging because it does not depend on an invasive procedure to obtain tissue for pathological evaluation.

When used with suitable labels or other appropriate detectable biomolecule or chemicals, the binding protein and/or antibody of the invention is particularly useful for *in vitro* and *in vivo* diagnostic and prognostic applications.

Labels for use in immunoassays are generally known to those skilled in the art and include enzymes, radioisotopes, and fluorescent, luminescent and chromogenic substances, including colored particles such as colloidal gold or latex beads. Suitable immunoassays include enzyme-linked immunosorbent assays (ELISA). Various types of labels and methods of conjugating the labels to the binding protein and/or the antibodies of the invention are well known to those skilled in the art, such as the ones set forth below.

As used herein, the term "an oncogenic disorder associated with expression of ligand independent activated form of cMet" is intended to include diseases and other disorders in which the presence of high levels or abnormally low levels of ligand independent activated form of cMet (aberrant) in a subject suffering from the disorder has been shown to be or is suspected of being either responsible for the pathophysiology of the disorder or a factor that contributes to a worsening of the disorder. Alternatively, such disorders may be evidenced, for example, by an increase in the levels of ligand independent activated form of cMet on the cell surface that results in an increased tyrosine autophosphorylation of cMet in the affected cells or tissues of a subject suffering from the disorder. The increase in ligand independent activated form of cMet

levels may be detected, for example, using the antibody 205A5 or 227D3 of the invention. Moreover, it refers to cells which exhibit relatively autonomous growth, so that they exhibit an aberrant growth phenotype characterized by a significant loss of control of cell proliferation. Alternatively, the cells may express normal levels of ligand independent activated form of cMet but are marked by abnormal proliferation.

In certain embodiments, "increased expression" as it relates to ligand independent activated form of cMet refers to protein or gene expression levels that demonstrate a statistically significant increase in expression (as measured by RNA expression or protein expression) relative to a control.

More particularly, it is considered the use of a binding protein or an antibody, or a functional fragment or derivative thereof, according to the invention as described, for diagnosing *in vitro* an oncogenic disorder associated with expression of ligand independent activated form of cMet, or determining *in vitro* the prognosis for developing an oncogenic disorder associated with expression of ligand independent activated form of cMet.

Another broad aspect in accordance with the invention concerns a method of diagnosing pathological hyperproliferative oncogenic disorder or a susceptibility to a pathological condition associated with expression of ligand independent activated form of cMet, in a subject comprising determining the presence or absence of ligand independent activated form of cMet bearing cells in a sample, and diagnosing a pathological condition or susceptibility to a pathological condition based on the presence or absence of said ligand independent activated form of cMet bearing cells. The diagnostic uses of the binding protein or the antibody of the invention comprise primary tumors, cancers and metastases. The binding protein or the antibody can be present in the form of an immunoconjugate or of a labeled binding protein/antibody so as to obtain a detectable and/or quantifiable signal.

More particularly, an preferred subject in accordance with the invention is a process of detecting *in vitro* the presence and/or the location of a ligand independent activated form of cMet expressing tumor in a subject, wherein said process comprises the steps of a) contacting a sample from the subject with a binding protein or an antibody, or a functional fragment or derivative thereof, according to the invention, and

b) detecting the binding of said binding protein or antibody with the sample. Another aspect of the subject is the follow-up of ligand independent activated form of cMet expression as a response to a cMet targeted therapy during clinical trials, and more particularly when the downregulation and or degradation of the ligand independent activated form of cMet is one of the component of the mechanism of action of the tested compound.

As will be apparent to the skilled artisan, the detection of the binding of the binding protein or the antibody of the invention may be revealed by various assays. Although any means for carrying out the assays is compatible with the invention, it can be mentioned, as examples, FACS, ELISA or IHC.

As used herein, the term "sample" is intended to mean any biological fluid, cell, tissue, organ or portion thereof, that includes or potentially includes a neoplastic cell, such as a cell from the colon, gastric, rectum, breast, ovary, prostate, kidney, lung, blood, brain or other organ or tissue that contains or is suspected to contain a neoplastic cell. The term includes samples present in an individual as well as samples obtained or derived from the individual. For example, a sample can be a histologic section of a specimen obtained by biopsy, or cells that are placed in or adapted to tissue culture. A sample further can be a subcellular fraction or extract, or a crude or substantially pure nucleic acid molecule or protein preparation.

Clinical sample is intended to encompass a variety of sample types obtained from a subject and useful in the procedure of the invention, such as for example, a diagnostic or monitoring test of determining or detecting ligand independent activated form of cMet, expression levels. The definition encompasses solid tissue samples obtained by surgical removal, a pathology specimen, an archived sample, or a biopsy specimen, tissue cultures or cells derived therefrom and the progeny thereof, and sections or smears prepared from any of these sources. Non-limiting examples are samples obtained from breast tissue, lymph nodes, colon, pancreas, prostate etc. The definition also encompasses liquid samples of biologic origin, and may refer to either the cells or cell fragments suspended therein, or to the liquid medium and its solutes.

Another aspect in accordance with the invention relates to a process of determining *in vitro* the expression level of ligand independent activated form of cMet, in a cMet expressing tumor from a subject, wherein said process comprises the steps of

a) contacting a sample from the subject with a binding protein or an antibody, or a functional fragment or derivative thereof, according to the invention, and b) quantifying the level of binding protein or antibody binding to ligand independent activated form of cMet in said sample.

As will be apparent to the skilled artisan, the level of binding protein or antibody binding to ligand independent activated form of cMet may be quantified in a number of ways such as by various assays. Although any means for carrying out the assays is compatible with the invention, a preferred method brings into play immunoenzymatic processes according to the ELISA technique, by immunofluorescence, by immunohistochemistry or radio-immunoassay (RIA) technique or equivalent.

In a preferred embodiment of the process of the invention the expression level of ligand independent activated form of cMet is measured by immunohistochemistry (IHC).

Preferably, the biological sample is formed by a biological fluid, such as serum, whole blood, cells, a tissue sample or biopsies of human origin. The sample, may for example include, biopsied tissue, which can be conveniently assayed for the presence of a pathological hyperproliferative oncogenic disorder associated with expression of ligand independent activated form of cMet.

Once a determination is made of the amount of ligand independent activated form of cMet present in the test sample, the results can be compared with those of control samples, which are obtained in a manner similar to the test samples but from individuals that do not have or present with a hyperproliferative oncogenic disorder associated with expression of ligand independent activated form of cMet. If the level of the ligand independent activated form of cMet is significantly elevated in the test sample, it may be concluded that there is an increased likelihood of the subject from which it was derived has or will develop said disorder.

The invention relates, more particularly, to a process of diagnosing *in vitro* a ligand independent activated form of cMet expressing tumor or determining *in vitro* the prognosis for developing a ligand independent activated form of cMet expressing tumor in a subject, wherein said process comprises the steps of a) determining the expression level of ligand independent activated form of cMet, as above described, and b) comparing the expression level of step a) with a reference expression level of ligand

independent activated form of cMet, from normal tissue or a non expressing ligand independent activated form of cMet tissue.

"Diagnosing" a disease as used in the application is intended to include, for example, diagnosing or detecting the presence of a pathological hyperproliferative oncogenic disorder associated with or mediated by expression of ligand independent activated form of cMet, monitoring the progression of the disease, and identifying or detecting cells or samples that are indicative of a disorder associated with the expression of ligand independent activated form of cMet.

"Prognosis" as used in this application means the likelihood of recovery from a disease or the prediction of the probable development or outcome of a disease. For example, if a sample from a subject is positive for staining with the binding protein or the antibody of the invention, then the "prognosis" for that subject is better than if the sample was negative for ligand independent activated form of cMet staining. Samples may be scored for ligand independent activated form of cMet expression levels on an appropriate scale as it will be more detailed hereinafter.

However another aspect of the invention is also related to the monitoring of ligand independent activated form of cMet expression for therapeutic compounds that induce a degradation of cMet as one of their mechanism of action. In that case following ligand independent activated form of cMet expression on cell membrane could be a critical tool to evaluate the efficacy of the treatment during clinical trials and "personalized" therapies.

The expression level of ligand independent activated form of cMet is advantageously compared or measured in relation to levels in a control cell or sample also referred to as a "reference level" or "reference expression level". "Reference level", "reference expression level", "control level" and "control" are used interchangeably in the specification. Broadly speaking, a "control level" means a separate baseline level measured in a comparable control cell, which is generally disease or cancer free. It may be from the same individual or from another individual who is normal or does not present with the same disease from which the diseased or test sample is obtained. Within the context of the present invention, the term "reference level" refers to a "control level" of expression of ligand independent activated form of cMet used to evaluate a test level of expression of ligand independent activated form of cMet in a

cancer cell-containing sample of a patient. For example, when the level of ligand independent activated form of cMet in the biological sample of a patient are higher than the reference level of ligand independent activated form of cMet, the cells will be considered to have a high level of expression, or overexpression, of ligand independent activated form of cMet. The reference level can be determined by a plurality of methods. Expression levels may thus define ligand independent activated form of cMet bearing cells or alternatively the level of expression of ligand independent activated form of cMet independent of the number of cells expressing ligand independent activated form of cMet. Thus the reference level for each patient can be proscribed by a reference ratio of ligand independent activated form of cMet, wherein the reference ratio can be determined by any of the methods for determining the reference levels described herein.

For example, the control may be a predetermined value, which can take a variety of forms. It can be a single cut-off value, such as a median or mean. The "reference level" can be a single number, equally applicable to every patient individually, or the reference level can vary, according to specific subpopulations of patients. Thus, for example, older men might have a different reference level than younger men for the same cancer, and women might have a different reference level than men for the same cancer. Alternatively, the "reference level" can be determined by measuring the level of expression of ligand independent activated form of cMet in non-oncogenic cancer cells from the same tissue as the tissue of the neoplastic cells to be tested. As well, the "reference level" might be a certain ratio of ligand independent activated form of cMet in the neoplastic cells of a patient relative to the ligand independent activated form of cMet levels in non-tumor cells within the same patient. The "reference level" can also be a level of ligand independent activated form of cMet of *in vitro* cultured cells, which can be manipulated to simulate tumor cells, or can be manipulated in any other manner which yields expression levels which accurately determine the reference level. On the other hand, the "reference level" can be established based upon comparative groups, such as in groups not having elevated ligand independent activated form of cMet levels and groups having elevated ligand independent activated form of cMet levels. Another example of comparative groups would be groups having a particular disease, condition or symptoms and groups without the disease. The predetermined value can be arranged,

for example, where a tested population is divided equally (or unequally) into groups, such as a low-risk group, a medium-risk group and a high-risk group or into quandrants or quintiles, the lowest quandrant or quintile being individuals with the lowest risk or highest amount of ligand independent activated form of cMet and the highest quandrant or quintile being individuals with the highest risk or lowest amount of ligand independent activated form of cMet.

The reference level can also be determined by comparison of the level of ligand independent activated form of cMet in populations of patients having the same cancer. This can be accomplished, for example, by histogram analysis, in which an entire cohort of patients are graphically presented, wherein a first axis represents the level of ligand independent activated form of cMet, and a second axis represents the number of patients in the cohort whose tumoral cells express ligand independent activated form of cMet at a given level. Two or more separate groups of patients can be determined by identification of subsets populations of the cohort which have the same or similar levels of ligand independent activated form of cMet. Determination of the reference level can then be made based on a level which best distinguishes these separate groups. A reference level also can represent the levels of two or more markers, one of which is ligand independent activated form of cMet. Two or more markers can be represented, for example, by a ratio of values for levels of each marker.

Likewise, an apparently healthy population will have a different 'normal' range than will have a population which is known to have a condition associated with expression of ligand independent activated form of cMet. Accordingly, the predetermined value selected may take into account the category in which an individual falls. Appropriate ranges and categories can be selected with no more than routine experimentation by those of ordinary skill in the art. By "elevated", "increased" it is meant high relative to a selected control. Typically the control will be based on apparently healthy normal individuals in an appropriate age bracket.

It will also be understood that the controls according to the invention may be, in addition to predetermined values, samples of materials tested in parallel with the experimental materials. Examples include tissue or cells obtained at the same time from the same subject, for example, parts of a single biopsy, or parts of a single cell sample from the subject.

In the clinical diagnosis or monitoring of patients with a ligand independent activated form of cMet mediated diseases, the detection of ligand independent activated form of cMet expressing cells or an increase in the levels of ligand independent activated form of cMet, in comparison to the levels in a corresponding biological sample from a normal subject or non-cancerous tissue is generally indicative of a patient with or suspected of presenting with a ligand independent activated form of cMet mediated disorder.

In accordance with the above, the invention provides for a method for predicting susceptibility to cancer comprising detecting the expression level of ligand independent activated form of cMet, in a tissue sample, its presence indicating susceptibility to cancer, wherein the degree of ligand independent activated form of cMet expression correlates to the degree of susceptibility. Thus, in specific embodiments, the expression of ligand independent activated form of cMet in, for example, prostate tissues, osteosarcomas tissue, lung tissue, pancreatic tissue, colon tissue, breast tissue, glyoblastoma tissue, ovarian tissues, or any other tissue suspected of cells expressing ligand independent activated form of cMet is examined, with the presence of ligand independent activated form of cMet in the sample providing an indication of cancer susceptibility or the emergence or existence of a tissue specific tumor.

A method for evaluating tumor aggressiveness is also provided. In one embodiment, a method for observing the progression of a malignancy in an individual over time comprises determining the level of ligand independent activated form of cMet expressed by cells in a sample of the tumor, comparing the level so determined to the level of ligand independent activated form of cMet expressed in an equivalent tissue sample taken from the same individual at a different time, wherein the degree of ligand independent activated form of cMet expression in the tumor sample over time provides information on the progression of the cancer.

In yet another embodiment, the application provides methods for determining the appropriate therapeutic protocol for a subject. Specifically, the binding protein or the antibodies of the invention will be very useful for monitoring the course of amelioration of malignancy in an individual, especially in those circumstances where the subject is being treated with a cMet binding protein or antibody that does not compete with the binding protein or the antibodies of the invention for binding to ligand independent

activated form of cMet. The presence or absence or a change in the level of ligand independent activated form of cMet in accordance with the invention may be indicative that the subject is likely to have a relapse or a progressive, or a persistent cancer associated with ligand independent activated form of cMet. Thus, by measuring an increase in the number of cells expressing ligand independent activated form of cMet or changes in the concentration of ligand independent activated form of cMet present in various tissues or cells, it is possible to determine whether a particular therapeutic regimen aimed at ameliorating a malignancy associated with ligand independent activated form of cMet is effective.

Another subject of the invention is an *in vivo* method of imaging an oncogenic disorder associated with expression of ligand independent activated form of cMet. For example, such a method can be used on a patient presenting symptoms of an oncogenic disorder. If the patient has, for example increased expression levels of ligand independent activated form of cMet, then the patient is likely suffering from a cancerous disorder. As well, the method can be useful for monitoring progression and/or response to treatment in patients who have been previously diagnosed with a ligand independent activated form of cMet mediated cancer. In accordance with the above objective, the invention provides an *in vivo* imaging reagent comprising a binding protein or an antibody according to the invention, or a functional fragment or derivative thereof, preferably labeled, especially radiolabeled, and its use in medical imaging. Thus, a general method in accordance with the invention works by administering to a patient an imaging-effective amount of an imaging reagent such as the above described binding protein or antibody which is labeled and a pharmaceutically effective carrier and then detecting the agent after it has bound to ligand independent activated form of cMet present in the sample. In certain embodiments, the method works by administering an imaging-effective amount of an imaging agent comprising a targeting moiety and an active moiety. The imaging agent is administered in an amount effective for diagnostic use in a mammal such as a human and the localization and accumulation of the imaging agent is then detected. The localization and accumulation of the imaging agent may be detected by radionucleide imaging, radioscintigraphy, nuclear magnetic resonance imaging, computed tomography, positron emission tomography, computerized axial

tomography, X-ray or magnetic resonance imaging method, fluorescence detection, and chemiluminescent detection.

In regards to the development of targeted antitumoral therapy, the diagnosis with immunohistological technics gives, *in situ*, information on the receptor expression level and thus enable to select patients susceptible to be treated following the expression level of receptors needed for such a treatment.

For immunotherapy using monoclonal antibodies, the response to the treatment depending of the receptor targeted expression level as treatment with trastuzumab where determination of Her2 overexpression in breast carcinoma is now of major clinical importance with the advent of the humanised anti-Her2 monoclonal antibody trastuzumab. Demonstration of Her2 overexpression is a prerequisite for treatment with trastuzumab as it acts by specifically targeting Her2 overexpressing carcinoma cells. Accurate testing for Her2 aims to ensure that costly and potentially toxic trastuzumab treatment is not given to patients with non-overexpessing tumours and that every patient who might benefit from trastuzumab receives appropriate treatment.

The teaching with trastuzumab concerning the patient selection that overexpressed Her2 showed the benefit to determine the expression level of receptor when using a therapy with a monoclonal antibody and to develop, in the same time than a therapeutic monoclonal antibody, a monoclonal antibody which can be used for the patient selection.

As a consequence, the invention relates to a process of determining *in vitro* the ligand independent activated form of cMet status of a tumor of a subject, wherein said process comprises the steps of a) determining the expression level of ligand independent activated form of cMet, as above described, b) scoring said tumor for ligand independent activated form of cMet expression level, and c) comparing said scoring to that obtained from a control sample.

"Ligand independent activated form of cMet status" within the meaning of the invention, relates to the classification of tumor to a ligand independent activated form of cMet positive [ligand independent activated form of cMet (+)] or ligand independent activated form of cMet negative [ligand independent activated form of cMet (-)] class based on the determination of the expression level of the ligand independent activated form of *cMet* gene as measured by any methods such as fluorescence *in situ*

hybridization (FISH), chromogenic *in situ* hybridization (CISH), gene chip or other methods known by the man skilled in the art.

In a preferred embodiment, the antibody for diagnostic have to be to able to bind the targeted receptor when tissue samples are formalin fixed and paraffin embedded.

More particularly, the ligand independent activated form of cMet expression level is measured by imunohistochemistry (IHC).

As an example, samples may be scored for ligand independent activated form of cMet expression levels on a scale from 0-3^+ for levels of binding protein or antibody staining, where 0 is negative and 1^+-3^+ represents positive staining at four semiquantitative steps of increasing intensity. Scores 1^+-3^+ can be recoded as positive because each positive score may be associated with significantly reduced risk for relapse and fatal disease when compared to score 0 (negative), but increasing intensity among the positive scores may provide additional risk reduction. Any conventional hazard analysis method may be used to estimate the prognostic value of ligand independent activated form of cMet. Representative analysis methods include Cox regression analysis, which is a semiparametric method for modeling survival or time-to-event data in the presence of censored cases (Hosmer and Lemeshow, 1999; Cox, 1972). In contrast to other survival analyses, e.g. Life Tables or Kaplan-Meyer, Cox allows the inclusion of predictor variables (covariates) in the models. Using a convention analysis method, e.g., Cox one may be able to test hypotheses regarding the correlation of ligand independent activated form of cMet expression status of in a primary tumor to time-to-onset of either disease relapse (disease-free survival time, or time to metastatic disease), or time to death from the disease (overall survival time). Cox regression analysis is also known as Cox proportional hazard analysis. This method is standard for testing the prognostic value of a tumor marker on patient survival time. When used in multivariate mode, the effect of several covariates are tested in parallel so that individual covariates that have independent prognostic value can be identified, i.e. the most useful markers. The term positive or negative "ligand independent activated form of cMet status" [also referred as ligand independent activated form of cMet (+) or ligand independent activated form of cMet (-)] of tumors refers to scores 0 or scores 1^+-3^+, respectively.

A sample may be "scored" during the diagnosis or monitoring of cancer. In its simplest form, scoring may be categorical negative or positive as judged by visual

examination of samples by immunohistochemistry. More quantitative scoring involves judging the two parameters intensity of staining and the proportion of stained ("positive") cells that are sampled. Based on these two parameters numbers may be assigned that reflect increasing levels of positive staining. Allred et al (Allred, Harvey et al. 1998) have described one way of achieving this, which involved scoring both parameters on a scale from 0 (negative) to 3^+, and summarizing the scores of the individual parameters to an overall score. This results in a scale with possible scores of 0, 2, 3, 4, 5, 6, 7 or 8 (Payne et al. Predictive markers in breast cancer – the present. Histopathology 2008, 52, 82-90) (Note that a score of 1 is not possible on Allred's scale). A somewhat simpler scoring method integrates the intensity of nuclear staining and the proportion of cells that display stained nuclei into a combined scale from 0 to 3^+. Either scoring method may be applied to scoring intensity and proportion of staining of activated Stat5 in the cell nuclei. The terms positive or negative "ligand independent activated form of cMet status" of tumors used in the present description refers to levels of expression of ligand independent activated form of cMet that correspond to scores 0 or 1^+-3^+ on the simplified scale, respectively.

Generally, the results of a test or assay according to the invention can be presented in any of a variety of formats. The results can be presented in a qualitative fashion. For example, the test report may indicate only whether or not a particular polypeptide was detected, perhaps also with an indication of the limits of detection. The results may be presented in a semi-quantitative fashion. For example, various ranges may be defined, and the ranges may be assigned a score (e.g., 1^+ to 3^+) that provides a certain degree of quantitative information. Such a score may reflect various factors, e.g., the number of cells in which ligand independent activated form of cMet is detected, the intensity of the signal (which may indicate the level of expression of ligand independent activated form of cMet or ligand independent activated form of cMet bearing cells), etc. The results may be presented in a quantitative fashion, e.g., as a percentage of cells in which the polypeptide (ligand independent activated form of cMet) is detected, as a protein concentration, etc. As will be appreciated by one of ordinary skill in the art, the type of output provided by a test will vary depending upon the technical limitations of the test and the biological significance associated with detection of the polypeptide. For example, in the case of certain polypeptides a purely qualitative output (e.g., whether or

not the polypeptide is detected at a certain detection level) provides significant information. In other cases a more quantitative output (e.g., a ratio of the level of expression of the polypeptide in the sample being tested versus the normal level) is necessary.

In a more preferred embodiment, scoring of ligand independent activated form of cMet expression level is graded from 0 to 3^+, based on an assessment of the intensity of the reaction product and the percentage of positive cells. For more clarity, table 4 hereinafter summarizes these parameters. Only complete circumferential membranous reactivity of the invasive tumour should be considered and often resembled a "chicken wire" appearance. Under current guidelines, samples scored as borderline (score of 2^+ or more) for ligand independent activated form of cMet IHC must be considered as ligand independent activated form of cMet (+) and are required to undergo further assessment. The IHC analysis should be rejected, and either repeated or tested by FISH or any other method if, as non limitative example, controls are not as expected, artifacts involve most of the sample and the sample has strong membranous positivity of normal breast ducts (internal controls) suggesting excessive antigen retrieval.

Table 4

status	IHC description
0	No reactivity or membranous reactivity in less than 10% of tumour cells
1^+	Faint/barely perceptible membranous reactivity is detected in more than 10% of tumour cells. The cells are immunoreactive only in part of the membrane.
2^+	Weak to moderate complete membranous reactivity is seen in more than 10% of tumour cells.
3^+	Strong complete reactivity is seen in more than 10% of tumour cells.

In a more preferred embodiment of the process according to the invention, said scoring comprises using an appropriate scale based on two parameters which are the intensity of the staining and the percentage of positive cells.

In a preferred embodiment, the process according to the invention, refers to an appropriate scale is a scale of 0 to 3^+ wherein no membranous reactivity of tumor cells is scored 0, and strong complete reactivity in more than 10% of tumor cells is scored 3^+.

In more details, as above described, said appropriate scale is a scale of 0 to 3 wherein no membranous reactivity of tumor cells is scored 0; faint perceptible membranous reactivity in more than 10% of tumor cells is scored 1^+; weak to moderate complete membranous reactivity in more than 10% of tumor cells is scored 2^+; and strong complete reactivity in more than 10% of tumor cells is scored 3^+.

In a particular aspect of the invention, a tumor is ligand independent activated form of cMet (+) with a score of 2^+.

In a particular aspect of the invention, a tumor is ligand independent activated form of cMet (+) with a score of 3^+.

In another particular aspect of the invention, a tumor is ligand independent activated form of cMet (+) with a score of 2^+ or 3^+.

According to the invention, it is also described a process of determining whether an oncogenic disorder is susceptible to treatment with a anti-ligand independent activated form of cMet binding protein or antibody, or a fragment or derivative thereof, wherein said process comprises the steps of (a) determining *in vitro* the ligand independent activated form of cMet status of a tumor of a subject as above described, and (b) determining that, if the status is ligand independent activated form of cMet (+), the oncogenic disorder is susceptible to treatment with an anti-ligand independent activated form of cMet binding protein or antibody, or a fragment or derivative thereof.

In another aspect of the invention, it is considered a kit useful for such diagnosing or prognosing process, said kit comprising the binding protein and/or the antibody of the invention.

As a matter of convenience, a packaged combination of reagents in predetermined amounts with instructions for performing the diagnostic assay, e.g. kits are also within the scope of the invention. The kit contains the binding proteins or antibodies for detection and quantitation of ligand independent activated form of cMet *in vitro*, e.g. in an ELISA or a Western blot. The binding protein or the antibody of the present invention can be provided in a kit for detection and quantitation of ligand independent activated form of cMet *in vitro*, e.g. in an ELISA or a Western blot. Where the binding protein or the antibody is labeled with an enzyme, the kit will include substrates and cofactors required by the enzyme (e.g., a substrate precursor which provides the detectable chromophore or fluorophore). In addition, other additives may

be included such as stabilizers, buffers (e.g., a block buffer or lysis buffer) and the like. Such a kit may comprise a receptacle being compartmentalized to receive one or more containers such as vials, tubes and the like, such containers holding separate elements of the invention. For example, one container may contain a first binding protein or antibody bound to an insoluble or partly soluble carrier. A second container may contain soluble, detectably-labeled second binding protein or antibody, in lyophilized form or in solution. The receptacle may also contain a third container holding a detectably labeled third binding protein or antibody in lyophilized form or in solution. A kit of this nature can be used in the sandwich assay of the invention. The label or package insert may provide a description of the composition as well as instructions for the intended *in vitro* or diagnostic use.

The relative amounts of the various reagents may be varied widely to provide for concentrations in solution of the reagents which substantially optimize the sensitivity of the assay. Particularly, the reagents may be provided as dry powders, usually lyophilized, including excipients which on dissolution will provide a reagent solution having the appropriate concentration.

In yet a further aspect of the invention, binding proteins, antibodies or binding fragments thereof as detailed herein are provided labeled with a detectable moiety, such that they may be packaged and used, for example, in kits, to diagnose or identify cells having the aforementioned antigen. Non-limiting examples of such labels include fluorophores such as fluorescein isothiocyanate; chromophores, radionuclides, or enzymes. Such labeled antibodies or binding fragments may be used for the histological localization of the antigen, ELISA, cell sorting, as well as other immunological techniques for detecting or quantifying ligand independent activated form of cMet, and cells bearing this antigen, for example.

Kits are also provided that are useful as a positive control for apoptosis assays, for purification or immunoprecipitation of ligand independent activated form of cMet from cells. For isolation and purification of ligand independent activated form of cMet, the kit can contain the antibodies described herein or antigen binding fragments thereof coupled to beads (e.g., sepharose beads). Kits can be provided which contain the binding protein or the antibodies for detection and quantitation of ligand independent activated form of cMet *in vitro*, e.g. in an ELISA or a Western blot. As with the article

of manufacture, the kit comprises a container and a label or package insert on or associated with the container. The container holds a composition comprising at least one anti-ligand independent activated form of cMet binding protein or antibody or binding fragment thereof of the invention. Additional containers may be included that contain, e.g., diluents and buffers, control antibodies. The label or package insert may provide a description of the composition as well as instructions for the intended *in vitro* or diagnostic use.

More particularly, the invention concerns a kit for the determination of the ligand independent activated form of cMet status of a tumor by any method known by the man skilled in the art. In a preferred embodiment, as it will be described in the example, the invention relates to a kit for the determination of the ligand independent activated form of cMet status of a tumor by IHC methods.

In a particular embodiment, the invention consists in a kit comprising at least a binding protein or an antibody, or a functional fragment or derivative thereof, as above describes, said binding protein or antibody being labeled.

It must be understood that any labeling method can be used by the man skilled in the art such as, for example, the use of labels above mentioned.

In a preferred embodiment, the kit according to the invention, useful for detecting *in vitro* the presence and/or the location of a ligand independent activated form of cMet expressing tumor in a subject, further comprises a reagent useful for detecting the extent of binding between the said labeled binding protein or antibody and ligand independent activated form of cMet.

In another preferred embodiment, the kit of the invention useful for determining *in vitro* the expression level of ligand independent activated form of cMet in a ligand independent activated form of cMet expressing tumor, further comprises a reagent useful for quantifying the level of binding between the said labeled binding protein or antibody and ligand independent activated form of cMet.

In still another embodiment, the kit according to the invention useful for determining *in vitro* the ligand independent activated form of cMet status of a tumor, further comprises:

a) a reagent useful for detecting the extent of binding between the said labeled binding protein or antibody and ligand independent activated form of cMet; and

b) positive and negative control samples useful for the scoring the ligand independent activated form of cMet expression level.

Said kit for determining *in vitro* the ligand independent activated form of cMet status of a tumor can further comprise:

i) a second labeled polyclonal antibody specific to murine antibodies;

ii) a reagent useful for detecting the extent of binding between the said second labeled antibody and murine antibodies to the ligand independent activated form of cMet; and

iii) positive and negative control samples useful for the scoring the ligand independent activated form of cMet expression level.

The invention also comprises a binding protein, or a functional fragment or derivative thereof including antibody, which cross-competes for binding to the ligand independent activated form of cMet with a binding protein according to the invention.

The invention also comprises a binding protein, or a functional fragment or derivative thereof including antibody, which cross-competes for binding to the ligand independent activated form of cMet with an antibody according to the invention.

In another embodiment, the invention concerns also the use of a binding protein or an antibody according to the invention, or a functional fragment or derivative thereof, for the identification of binding proteins, including antibodies, able to specifically bind to the ligand independent activated form of cMet.

More particularly, in a preferred embodiment, it is described a process of identifying a binding partner to the ligand independent activated form of cMet, which comprises the steps of:

a) contacting the ligand independent activated form of cMet with a binding protein or an antibody according to the invention, or a functional fragment or derivative thereof;

b) contacting the complex of a) with a compound library,

c) identifying a compound which disrupts the complex of a).

The invention also comprises a process for purifying the ligand independent activated form of cMet, wherein said process comprises the following steps:

a) incubating a binding protein or an antibody according to the invention, or a functional fragment or derivative thereof with a sample under conditions to allow

specific binding of said binding protein or antibody and the ligand independent activated form of cMet; and

b) separating the binding protein or antibody from the sample and obtaining the purified ligand independent activated form of cMet.

In another particular aspect, the invention comprises a complex formed by the binding of a binding protein according to the invention, or a functional fragment or derivative thereof and the ligand independent activated form of cMet.

Similarly, the invention also comprises a complex formed by the binding of an antibody according to the invention, or a functional fragment or derivative thereof and the ligand independent activated form of cMet.

In another embodiment, the invention deals with the use of a binding protein or an antibody according to the invention, or a functional fragment or derivative thereof, as a vehicule intending for the specific targeting of a biologically active compound to cells expressing the ligand independent activated form of cMet. More particularly, said biological active compound is selected from the group consisting of chemotherapeutics, radioisotopes or toxins.

In still another embodiment, the invention consists of a process for generating an antibody, or a functional fragment or derivative thereof, which i) specifically binds to the ligand independent activated form of cMet, but ii) does not bind to the non activated and/or ligand dependent activated form(s) of cMet, said process being characterized in that it comprises the steps of:

a) immunizing an animal with transfected or tumoral cell lines expressing cMet protein or a fragment thereof;

b) removing antibody-producing cells from the animal;

c) fusing said antibody-producing cells with myeloma cells so as to obtain hybridoma cells;

d) carrying out screening assays so as to select hybridoma cells which produce antibody which i) specifically binds to the ligand independent activated form of cMet, but ii) does not bind to the non activated and/or ligand dependent activated form(s) of cMet;

e) culturing the selected hybridoma in a cell culture that produces the antibody; and

f) removing the antibody from the cell culture.

In a more preferred embodiment, the screening assay of the above described step d) consists of an IHC screening assay.

In another more preferred embodiment, said IHC screening assay comprises the steps of:

a) collecting tissue sections from tumors that express different forms of cMet,

b) performing IHC staining simultaneously on the different tissue sections of step a) using hybridoma cells of step c) of the process for generating an antibody, or a functional fragment or derivative thereof, which i) specifically binds to the ligand independent activated form of cMet, but ii) does not bind to the non activated and/or ligand dependant activated form(s) of cMet above described,

c) selecting hybridoma cells having a specific reactivity on tissue sections that express the ligand independent activated form of cMet and no reactivity on other tissue sections.

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

Figures 1A-1B: FACS recognition of cMet by the m205A5 (A) or the m227D3 (B) Mabs

Figure 2: Titration curves of m205A5 and m227D3 Mabs on the immobilized dimeric cMet protein.

Figures 3A-3C: m227D3 and m205A5 IHC pattern of recognition on paraffin embedded tumors (MCF7, U87MG and Hs746T).

Figures 4A-4C: IHC panel of recognition of the m227D3 and commercial anti cMet antibody 3D4 on paraffin embedded tumors (MCF7, U87MG, Hs746T, MKN45 and EBC-1).

Figure 5: HGF competition assay with the m205A5 or the m227D3 Mabs by ELISA.

Figure 6: Anti tumoral activity of the m224G11 on the Hs746T xenograft model.

Figure 7: *ex vivo* analysis of the cMet expression on Hs746T tumors of control mice and m224G11 treated mice by Western Blot analysis.

Figures 8A-8B: *ex vivo* analysis of the cMet expression on Hs746T tumors of control mice and m224G11 treated mice by IHC using the m205A5.

Figure 9: IHC staining of paraffin-embedded sections form liver tumor tissues expressing various levels of cMet.

Example 1: Generation of an antibody against ligand independent activated form of cMet that could be used for diagnostic purpose.

To generate anti-cMet antibodies 8 weeks old BALB/c mice were immunized either 3 to 5 times subcutaneously with a CHO transfected cell line that express cMet on its plasma membrane ($20x10^6$ cells/dose/mouse) or 2 to 3 times with a cMet extracellular domain fusion protein (10-15 µg/dose/mouse) (R&D Systems, Catalog # 358MT) or fragments of this recombinant protein mixed with complete Freund adjuvant for the first immunization and incomplete Freund adjuvant for the following one. Three days before cell fusion, mice were boosted i.p. or i.v. with the recombinant protein or fragments. Then spleens of mice were collected and fused to SP2/0-Ag14 myeloma cells (ATCC) and subjected to HAT selection. In general, for the preparation of monoclonal antibodies or their functional fragments, especially of murine origin, it is possible to refer to techniques which are described in particular in the manual "Antibodies" (Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor NY, pp. 726, 1988) or to the technique of preparation of hybridomas described by Kohler and Milstein (Nature, 256:495-497, 1975).

Obtained hybridomas were initially screened by ELISA on the dimeric cMet-Fc recombinant protein. Briefly, the recombinant human cMet protein was coated overnight at 4°C to Immulon II 96-well plates and, after a 1 h blocking step with a 0.5% gelatine solution, pure hybridoma supernatant was added for an additional 1 h at 37°C. Then plates were washed and a goat anti-mouse (Jackson) specific IgG HRP was added for 1 h at 37°C. Reaction development was performed using the TMB substrate solution. Then a second screen was performed by FACS analysis on A459 cell line, that express moderate to high levels of cMet, to be sure that the produced antibodies will be able to also recognize the native receptor on tumor cells. For that purpose $2x10^5$ cells were incubated with either 10µg/ml of m205A5, m227D3 or m10D9 (IgG1 isotype control Mab) for 20 min at 4°C. After 3 washing in phosphate-buffered saline (PBS)

supplemented with 1% BSA and 0.01% NaN_3, cells were incubated with secondary antibody Goat anti-mouse Alexa 488 (dilution 1/500) for 20 minutes at 4°C. After 3 additional washings in PBS supplemented with 1% BSA and 0.1% NaN_3, cells were analyzed by FACS (Facscalibur, Becton-Dickinson). At least 5000 cells were assessed to calculate the mean value of fluorescence intensity.

Table 5

Data from cytometry analysis (MFI) performed with the 205A5 and 227D3 Mabs on 5 tumoral human cell lines (ATCC)

	A549	**BXPC-3**	**MCF7**	**U87MG**	**HepG2**
Cells only	13.98	11.87	9.87	9.10	10.52
Secondary antibody	11.98	13.23	11.10	11.20	15.85
Isotype control	11.83	14.77	12.06	11.56	18.12
205A5	189.94	217.87	22.78	48.71	151.89
227D3	144.28	158.04	15.89	35.35	110.83

Positive hybridomas on this assay were amplified, cloned, isotyped and expanded. Then new hybridoma supernatants were collected, Their IgG content determined. Complementary cytometry analysis were performed on a panel of 5 human tumoral cell lines (A459, BXPC3, MCF7, U87MG, and HepG2). All these cell lines were provided by the ATCC. Data obtained are presented in Figure 1 and MFI values presented in Table 5.

Complementary experiments were done with purified 205A5 or 227D3 antibodies. First antibody titration on the dimeric cMet-Fc protein was performed. Titration curves are presented in Figure 2.

205A5 and 227D3 antibodies had fulfil the 2 criteria described above (i) cMet recognition on an ELISA test, (ii) binding on the native cMet expressed on the surface of human tumoral cell lines and were selected for further assays.

Mabs were selected for the final cMet recognition test on paraffin-embedded sections from tumor xenografts expressing cMet. For that evaluation, tumor sections from MCF7, U87-MG and Hs746T (tumors known to express variable levels of cMet)

xenografts were deparaffinized, rehydrated, and placed in Target Retrieval Buffer 1X (Dako S1699) in a boiling bath pre-warm at 98°C for heat-induced epitope retrieval at 98°C for 30 minutes and then for 30 additional minutes in the Target Retrieval Buffer. After 3 washes in Tris Buffer Saline-0.05% tween 20 (TBS-T) (Dako S3006), the endogenous peroxidase activity was blocked using Peroxidase Blocking Reagent (Dako K4007) for five minutes. Sections were washed with TBS-T and incubated with blocking reagent (UltraV block-TA-125UB- LabVision) for 5 minutes before addition of the cMet mouse monoclonal antibody to be tested (5 µg/ml). A mouse IgG1/kappa (5 µg/ml, X0931, Dako) was used as a negative control. Sections were then incubated for 2 hours at room temperature, washed with TBS-T and incubated with Envision Dual Link Peroxydase System (Dako K4061). Diaminobenzidine (DAB) peroxydase substrate was used for development of a brown reaction product.

Results shown in Figure 3 demonstrated that, as expected no staining was observed with an IgG1 isotype control (Figure 3 Panel A) and that both 227D3 (Figure 3 Panel B) and 205A (Figure 3 Panel C) were able to recognize cMet only on Hs746T xenograft tumors whereas all 3 tumors expressed various level of cMet. These first results suggest that both 205A5 and 227D3 antibodies recognize a particular form of cMet that is only expressed on amplified tumor cells.

To confirm the pattern of recognition of these Mabs, a panel of tumors from xenografted mice were tested for cMet expression using either 205A5, 227D3 or 3D4, a commercially available antibody from Invitrogen described for its recognition of cMet on IHC. As shown in Figure 4 Panel C, 3D4 recognized cMet in all tumor types. Using 227D3 (Panel A) or 205A5 (data not shown), a strong membranous staining was only observed on the 3 tumor types bearing a constitutively activated form of cMet (due to *cMet* amplification resulting in an over expression of cMet): Hs746T, MKN45 and EBC-1. As expected, no staining was observed with the mIgG1 isotype control (Figure 4 Panel B).

Example 2: HGF competition experiments performed in presence of the 205A5 or 227D3 antibodies.

To further characterize the diagnostic Mabs, HGF competition assays were performed. First reaction mixture comprising the cMet protein in presence or not of the

Mabs to be tested, were prepared on a separate saturated (0.5% gelatin in PBS 1X) plate. Serial 1: 2 dilutions (starting from 40 µg/ml over 12 columns) of murine antibodies (references and Mabs to study) were performed. Then 0.8 µg/ml of the rh cMet-Fc protein was added (RDSystems, ref. 358-MT/CF), except to the negative control line that contained only ELISA diluant (0.1% gelatin, 0.05% Tween 20 in PBS 1X). After homogenisation, the competition samples were loaded on HGF-coated plates with a 0.3 µg/ml rhHGF solution in PBS (RDSystems, ref. 294-HGN/CF). After an incubation and several washes, bound cMet proteins are detected using a goat anti-Human IgG-HRP (Jackson, ref. 109-035-098). Once bound, the TMB substrate was added to the plates. The reaction was stopped by addition of H_2SO_4 acid solution and the obtained optical densities read at 450 nm using a microplate reader instrument.

The experiment was carried out with 205A5 and 227D3 in presence or in absence of cMet-Fc recombinant protein. (see Figure 5). This experiment showed that 205A5 and 227D3 Mabs did not compete with the cMet binding on its immobilized ligand receptor.

Example 3: Antibodies specificity

m224G11 treatment of mice bearing Hs746T tumor induced a significant decrease of tumor growth (Figure 6). When tumor from this experiment were analysed, the anti-tumoral activity resulted in a decrease of Ki67 expression in m224G11 treated mice *vs* control mice (data not shown) and in a dramatic down-regulation of cMet expression demonstrated by western Blot analysis (Figure 7). To perform WB analysis, tumors were removed and quickly snap frozen in nitrogen. Then they were lysed and the same amount of protein was immunoblotted. Finally, cMet was revealed using the commercially available anti-Met-beta subunit 25H2 Mab from Cell Signalling.

When tumors from the same experiment were analysed by IHC using the 205A5, the expected down-regulation of cMet expression was observed in the treated mice, whereas all tumor cells of the control group were Met positive (Figure 8).

Indeed, as shown in Figure 8A, when tumors from control mice were stained using 205A5 Mab, a strong membranous and homogeneous staining was observed in tumor cells of all 3 mice. When tumors from m224G11 treated mice were stained with 205A5, a dramatic decrease of the membranous staining was observed (Figure 8B).

These data in agreement with the one previously observed by Western Blot analysis (Figure 7) demonstrate thet 205A5 recognize specifically cMet.

Example 4: Scoring tissues for ligand independent activated form of cMet expression with the m224G11 Mab

Using the protocol described above and summarized in Figure 9, a set of paraffin-embedded human tumor tissues, expressing variable levels of ligand independent activated form of cMet were stained with the m205A5 Mab.

Results shown in Figure 9 demonstrated, in hepatocellular carcinoma, that m205A5 was able to discriminate human tumors with variable levels of ligand independent activated form of cMet. Using this antibody, tumors could be scored as:

- 0: negative tumors in which no membrane staining or less than 10% membrane positive cell were observed,
- 1^+: barely perceptible staining in more than 10% of tumor cells,
- 2^+: Moderate complete membrane staining observed in more than 10 % tumor cells,
- 3^+: A strong complete staining of more than 10% of tumor cells.

PCT

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0-1	**Form PCT/RO/134 (SAFE) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)**	
0-1-1	Prepared Using	PCT Online Filing Version 3.5.000.225 MT/FOP 20020701/0.20.5.20
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1	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
1-1	**page**	22 ; 55
1-2	**line**	6-7,10 ; 7
1-3	**Identification of deposit**	
1-3-1	Name of depositary institution	CNCM Collection nationale de cultures de micro-organismes
1-3-2	Address of depositary institution	Institut Pasteur, 28, rue du Dr Roux, 75724 Paris Cedex 15, France
1-3-3	Date of deposit	18 November 2009 (18.11.2009)
1-3-4	Accession Number	CNCM I-4247
1-5	**Designated States for Which Indications are Made**	All designations
2	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
2-1	**page**	22-23 ; 55
2-2	**line**	20;1,6 ; 8-9
2-3	**Identification of deposit**	
2-3-1	Name of depositary institution	CNCM Collection nationale de cultures de micro-organismes
2-3-2	Address of depositary institution	Institut Pasteur, 28, rue du Dr Roux, 75724 Paris Cedex 15, France
2-3-3	Date of deposit	18 November 2009 (18.11.2009)
2-3-4	Accession Number	CNCM I-4246
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CLAIMS

1. A binding protein, or a functional fragment or derivative thereof, which on tumors i) specifically binds to the ligand independent activated form of cMet, characterized in that:

i) its does not bind to the non activated and/or ligand dependant activated form(s) of cMet; and, optionally,

ii) it does not block the binding of the ligand HGF to cMet; and, optionally,

iii) it interacts with the extra-cellular region of cMet between amino acid residues 1 and 950.

2. A binding protein, or a functional fragment or derivative thereof, according to claim 1, characterized in that it consists of an isolated antibody or a functional fragment or derivative thereof, selected from the group consisting of:

a) an antibody, or a functional fragment or derivative thereof, comprising:

- a light chain comprising the following three CDRs as defined according to IMGT, respectively CDR-L1 having the sequence SEQ ID No. 1, CDR-L2 having the sequence SEQ ID No. 2 and CDR-L3 having the sequence SEQ ID No.3; and
- a heavy chain comprising the following three CDRs as defined according to IMGT, respectively CDR-H1 having the sequence SEQ ID No. 4, CDR-H2 having the sequence SEQ ID No. 5 and CDR-H3 having the sequence SEQ ID No. 6, and

b) an antibody, or a functional fragment or derivative thereof, comprising

- a light chain comprising the following three CDRs as defined according to IMGT, respectively CDR-L1 having the sequence SEQ ID No. 9, CDR-L2 having the sequence SEQ ID No. 10 and CDR-L3 having the sequence SEQ ID No.11; and
- a heavy chain comprising the following three CDRs as defined according to IMGT, respectively CDR-H1 having the sequence SEQ ID No. 12, CDR-H2 having the sequence SEQ ID No. 13 and CDR-H3 having the sequence SEQ ID No. 14.

3. An antibody, or a functional fragment or derivative thereof, according to claim 2, characterized in that it selected from the group consisting of:

a) an antibody, or a functional fragment or derivative thereof, comprising a light chain variable domain of sequence having the amino acid sequence SEQ ID No. 7 and a heavy chain variable domain of sequence having the amino acid sequence SEQ ID No. 8; and

b) an antibody, or a functional fragment or derivative thereof, comprising a light chain variable domain of sequence having the amino acid sequence SEQ ID No. 15 and a heavy chain variable domain of sequence having the amino acid sequence SEQ ID No. 16.

4. A murine hybridoma capable of secreting an antibody according to claim 2 or 3, said murine hybridoma being selected from the hybridoma deposited at the CNCM, Institut Pasteur, Paris, on November 18, 2009 under the number I-4247 and the hybridoma deposited at the CNCM, Institut Pasteur, Paris, on November 18, 2009 under the number I-4246.

5. Monoclonal antibody derived from hybridoma I-4247 or I-4246 or a subclone thereof which on tumors i) specifically binds to the ligand independent activated form of cMet, but ii) does not bind to the non activated and/or ligand dependent activated form(s) of cMet.

6. An isolated nucleic acid, characterized in that it is chosen from the following nucleic acids:

a) a nucleic acid, DNA or RNA, coding for a binding protein as claimed in claim 1;

b) a nucleic acid, DNA or RNA, coding for an antibody as claimed in one of claims 2, 3 and 5;

c) a nucleic acid comprising a DNA sequence comprising the sequences SEQ ID Nos. 27 to 32 or 35 to 40;

d) a nucleic acid comprising a DNA sequence comprising the sequences SEQ ID Nos. 33, 34, 41 or 42;

e) the corresponding RNA nucleic acids of the nucleic acids as defined in c) or d); and

f) the complementary nucleic acids of the nucleic acids as defined in a), b), c) and d).

7. Use of a binding protein according to claim 1 or a monoclonal antibody according to one of claims 2, 3 and 5, or a functional fragment or derivative thereof, for the identification of the ligand independent activated form of cMet.

8. A process for differentiating between the ligand independent activated form of cMet and the others forms of cMet, including non activated or ligand dependent

activated forms of cMet, in a sample, which process comprises the steps of:

a) contacting said sample with a binding protein according to claim 1 or an antibody according to one of claims 2, 3 and 5, or a functional fragment or derivative thereof, and

b) detecting the binding of said binding protein or antibody with the sample.

9. Use of a binding protein according to claim 1 or an antibody according to one of claims 2, 3 and 5; or a functional fragment or derivative thereof, for diagnosing *in vitro* an oncogenic disorder associated with the ligand independent activation of cMet or determining by immunolabeling the prognosis for developing an oncogenic disorder associated with the ligand independent activation of cMet.

10. A process of detecting by immunolabeling the presence and/or the location of a ligand independent activated form of cMet expressing tumor in a subject, wherein said process comprises the steps of:

a) contacting a sample from the subject with a binding protein according to claim 1 or an antibody according to one of claims 2, 3 and 5, or a functional fragment or derivative thereof, and

b) detecting the binding of said binding protein or antibody with the sample.

11. A process of determining by immunolabeling the expression level of ligand independent activated form of cMet in a cMet expressing tumor from a subject, wherein said process comprises the steps of:

a) contacting a sample from the subject with a binding protein according to claim 1 or an antibody according to one of claims 2, 3 and 5, or a functional fragment or derivative thereof, and

b) quantifying the level of binding protein or antibody binding to ligand independent activated form of cMet in said sample.

12. A process according to claim 11, wherein the expression level of ligand independent activated form of cMet is measured by immunohistochemistry (IHC).

13. A process of diagnosing by immunolabeling a ligand independent activated form of cMet expressing tumor or determining by immunolabeling the prognosis for developing a ligand independent activated form of cMet expressing tumor in a subject, wherein said process comprises the steps of:

a) determining the expression level of ligand independent activated form of

cMet according to claim 11, and

b) comparing the expression level of step a) with a reference expression level of ligand independent activated form of cMet from normal tissue.

14. A process of determining by immunolabeling the ligand independent activated form of cMet status of a tumor of a subject, wherein said process comprises the steps of:

a) determining the expression level of ligand independent activated form of cMet according to claim 11,

b) scoring said tumor for ligand independent activated form of cMet expression level, and

c) comparing said scoring to that obtained from a control sample.

15. A process of determining whether an oncogenic disorder is susceptible to treatment with a anti-ligand independent activated form of cMet binding protein or antibody, or a fragment or derivative thereof, wherein said process comprises the steps of:

a) determining by immunolabeling the ligand independent activated form of cMet status of a tumor of a subject according to claim 11, and

b) determining that, if the status is ligand independent activated form of cMet(+), the oncogenic disorder is susceptible to treatment with an anti-ligand independent activated form of cMet binding protein or antibody, or a fragment or derivative thereof.

16. A kit comprising at least a binding protein according to claim 1 or an antibody according to one of claims 2, 3 and 5, or a functional fragment or derivative thereof, said binding protein or antibody being labeled.

17. A kit according to claim 16 for detecting by immunolabeling the presence and/or the location of a ligand independent activated form of cMet expressing tumor in a subject, said kit further comprising a reagent useful for detecting the extent of binding between the said labeled binding protein or antibody and ligand independent activated form of cMet.

18. A binding protein, or a functional fragment or derivative thereof including antibody, characterized in that it cross-competes for binding to the ligand independent activated form of cMet with a binding protein according to claim 1 or an antibody

according to one of claims 2, 3 and 5.

19. Use of a binding protein according to claim 1 or an antibody according to one of claims 2, 3 and 5, or a functional fragment or derivative thereof, for the identification of binding proteins, including antibodies, able to specifically bind to the ligand independent activated form of cMet.

20. A process of identifying a binding partner to the ligand independent activated form of cMet, characterized in that it comprises the steps of:

a) contacting the ligand independent activated form of cMet with a binding protein according to claim 1 or an antibody according to one of claims 2, 3 and 5, or a functional fragment or derivative thereof;

b) contacting the complex of a) with a compound library,

c) identifying a compound which disrupts the complex of a).

21. A process for purifying the ligand independent activated form of cMet, characterized in that it comprises the following steps:

a) incubating a binding protein according to claim 1 or an antibody according to one of claims 2, 3 and 5, or a functional fragment or derivative thereof with a sample under conditions to allow specific binding of said binding protein or antibody and the ligand independent activated form of cMet; and

b) separating the binding protein or antibody from the sample and obtaining the purified ligand independent activated form of cMet.

22. Complex formed by the binding of a binding protein according to claim 1 or an antibody according to one of claims 2, 3 and 5, or a functional fragment or derivative thereof and the ligand independent activated form of cMet.

23. Use of a binding protein according claim 1 or an antibody according to one of claims 2, 3 and 5, or a functional fragment or derivative thereof, as a vehicle intending for the specific targeting of a biologically active compound to cells expressing the ligand independent activated form of cMet.

24. A process for generating an antibody, or a functional fragment or derivative thereof, which i) specifically binds to the ligand independent activated form of cMet, but ii) does not bind to the non activated and/or ligand dependent activated form(s) of cMet, said process being characterized in that it comprises the steps of:

a) immunizing an animal with transfected or tumoral cell lines expressing cMet

protein or a fragment thereof;

b) removing antibody-producing cells from the animal;

c) fusing said antibody-producing cells with myeloma cells so as to obtain hybridoma cells;

d) carrying out screening assays so as to select hybridoma cells which produce antibody which i) specifically binds to the ligand independent activated form of cMet, but ii) does not bind to the non activated and/or ligand dependent activated form(s) of cMet;

e) culturing the selected hybridoma in a cell culture that produces the antibody; and

f) removing the antibody from the cell culture.

25. A process according to claim 24, characterized in that the screening assay of step d) consists of an IHC screening assay, said IHC screening assay preferably comprises the steps of:

a) collecting tissue sections from tumors that express different forms of cMet, b) performing IHC staining simultaneously on the different tissue sections of step a) using hybridoma cells of claim 24, step c),

c) selecting hybridoma cells having a specific reactivity on tissue sections that express the ligand independent activated form of cMet and no reactivity on other tissue sections.