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(54) Title: METHODS OF IDENTIFYING PATIENTS SUFFERING FROM LIVER CANCER WHO WILL MOST LIKELY BENEFIT FROM A TREATMENT WITH AN ANTAGONIST ANTI-FGFR4 ANTIBODY

(57) Abstract: The present invention relates to a method of identifying a patient suffering from a liver cancer who will most likely benefit from a treatment with an antagonist anti-FGFR4 antibody, this method relies on the conjoint determination of (i) the status of the single-nucleotide polymorphism (SNP) in codon 388 of FGFR4 on both alleles of the homologous chromosomes, and (ii) the status of activation of FGFR4. The invention also concerns a method of treating a patient with an antagonist anti-FGFR4 antibody, said patient being identified by the method of the invention as potentially benefiting from a treatment with an antagonist anti-FGFR4 antibody.



METHODS OF IDENTIFYING PATIENTS SUFFERING FROM LIVER CANCER WHO WILL MOST LIKELY BENEFIT FROM A TREATMENT WITH AN ANTAGONIST ANTI-FGFR4 ANTIBODY

FIELD OF THE INVENTION

The present invention relates to a method of identifying a patient suffering from a liver cancer who will most likely benefit from a treatment with an antagonist anti-FGFR4 antibody, this method relies on the conjoint determination of both (i) the status of the single-nucleotide polymorphism (SNP) in codon 388 of FGFR4 on both alleles of the homologous chromosomes, and (ii) the status of activation of FGFR4.

The invention also concerns a method of treating a patient with an antagonist anti-FGFR4 antibody, said patient being identified by the method of the invention as potentially benefiting from a treatment with an antagonist anti-FGFR4 antibody.

In a particular embodiment, the antagonist anti-FGFR4 antibody used for treating a patient suffering from a liver cancer comprises the six CDRs having the sequences SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10, respectively, or six CDRs the sequences of which differ from their corresponding respective sequences mentioned above at most by one or two amino acids.

BACKGROUND

Liver cancer is a frequent cancer worldwide, and one of the most common cause of cancer-related death. Liver cancer includes hepatocellular carcinoma (also known as HCC), which is the most common form of liver cancer, cholangiocarcinoma and hepatoblastoma. HCC often arises in patients afflicted by other underlying liver pathologies, including hepatitis and cirrhosis, which cause liver inflammation and chronic injury and regeneration of liver cells.

Standard treatment for liver cancer is surgical resection, when possible, and chemotherapy, chemoembolisation, or radiation therapy where surgery is not an option. Surgery is not always an option due to tumor size or location, advanced cirrhosis, and even with surgery, tumor recurrence/relapse complicates 70% of cases at 5 years post-resection. Chemotherapeutic treatments also appear to have reduced efficacy in liver cancer, possibly due to increased ability of liver cancer cells to efflux chemotherapeutic agents.

Recently, it has been shown that antagonist anti-fibroblast growth factor receptor 4 antibodies (hereinafter abbreviated as "anti-FGFR4 antibodies"), i.e. antibodies which specifically bind FGFR4 and thereby inhibit or block the activity or function of FGFR4, can be

useful for treating cancer, especially liver cancer, in particular by inhibiting tumor angiogenesis (see WO 2010/004204).

FGFR4 belongs to the fibroblast growth factor receptor family. FGFR4 possesses 802 amino acids (e.g. NCBI accession number NP_998812.1), and contains a signal peptide which spans amino acids at position 1 to 17, an extracellular domain composed of three immunoglobulin-like domains which form the ligand-binding domain, an acid box, a single transmembrane domain with 26 amino acids and an intracellular split tyrosine kinase.

When the extracellular portion of the protein interacts with its ligand, receptor dimerization is induced and leads to its activation, then setting in motion a phosphorylation cascade-mediated signaling pathway. This induction results in the autophosphorylation of the tyrosine kinase domain of FGFR4 and serves to initiate an intracellular signaling pathway dependent on the phosphorylation of other signaling proteins such as AKT, p44/42, JNK etc. This phosphorylation-mediated signaling varies according to the cell type and according to the coreceptors or the adhesion molecules present at the surface of the cell (Cavallaro *et al.*, Nat. Cell Biol. 3/7, 650-657, 2001; Stadler *et al.*, Cell. Signal. 18/6, 783-794. (2006); Lin *et al.*, J Biol Chem., 14:27277-84, 2007).

While several Fibroblast Growth Factors (FGF) can interact with FGFR4, such as FGF1 and FGF2, FGF19 has been identified as a ligand with high affinity exclusively for FGFR4 (Xie *et al.* Cytokine, 11:729-35, 1999), the binding of which to the receptor is heparin-dependent or heparan sulphate-dependent. FGF19 has been identified in adult animals, only in the hepatocytes and the small intestine.

It is known that the expression of a FGFR4 ligand (FGF-19) is implied in receptor activation by an autocrine loop in HCC. It has been demonstrated that ectopic expression of FGF19 in mice promotes hepatocyte proliferation, hepatocellular dysplasia, and neoplasia (Nicholes K *et al.*, Am J Pathol 160: 2295–2307, 2002). It has also been shown that a specific antibody directed against FGF19 abolished signaling mediated through FGFR4 in vitro, resulting in an inhibition of tumor xenografts in vivo and preventing hepatocellular carcinomas in FGF19 transgenic mice.

Somatic mutations of FGFR4 have been reported in different cancers, for instance FGFR4-activating mutations have been reported in human rhabdomyosarcomas (a childhood cancer originating from skeletal muscle) as promoting metastasis (J.G. Taylor VI *et al.*, J Clin Invest. 2009; 119(11):3395–3407). Amplification of FGFR4 has also been reported in breast and gynecological cancers.

A single nucleotide polymorphism (SNP) in FGFR4, having accession number rs351855 in the dbSNP database entry (for example available on the ncbi.nlm.nih.gov/snp

and ncbi.nlm.nih.gov/projects/SNP/ world wide web sites) (antisense brand, GTGGAGCGCCTGCCCTCGATACAGCC[C/T]GGCCAGCAGCAGGAGCACAGCCAAG; SEQ ID NO: 1), has been recently discovered in exon 9 of FGFR4 gene. This SNP is characterized by conversion of guanine to adenine in FGFR4 gene at position corresponding to position 1373 in FGFR4 mRNA having NCBI accession number NM_213647.1 (SEQ ID NO: 2).

This guanine to adenine conversion results in substitution of arginine for glycine at codon 388 (GGG → AGG) in the trans-membrane domain of FGFR4 (e.g. see NCBI accession number NP_998812.1:p.Gly388Arg). The FGFR4-Arg388 (abbreviated as FGFR4-R388) results in enhanced stability of the activated receptor (Ingvarsen *et al.*, Biol Chem.; 389(7):943-53, 2008). It was found that 40 to 50% of Caucasians carry at least one copy of this allele.

The FGFR4-R388 allele has been reported as being associated with poor outcomes and cancer aggressiveness in different cancers but no with predisposition to any cancer. FGFR4 arg388 allele represents an innocuous determinant in healthy individuals but predisposes cancer patients for significantly accelerated disease progression. However, the mechanisms by which this FGFR4 variant promotes enhanced oncogenesis remain unclear.

The inventors have now found that the genetic status of FGFR4 gene at codon 388 is crucial for the success of treatment of liver cancer with an antagonist anti-FGFR4 antibody, and that patients stratification based on their SNP in codon 388 of FGFR4 gene and their level of FGFR4 activation allows identifying patients who may benefit from a treatment with an antagonist anti-FGFR4 antibody, thus increasing the chance of success of the therapy.

DESCRIPTION OF THE INVENTION

The present invention arises from the finding by the inventors that the status of the single-nucleotide polymorphism (SNP) in codon 388 of FGFR4 on both alleles of the homologous chromosomes, and the activation status of FGFR4, when considered together, can be used as biomarkers for accurately selecting patients suffering from liver cancer who will most likely benefit from a treatment with an antagonist anti-FGFR4 antibody, i.e. patients who will likely be responsive to a treatment with an antagonist anti-FGFR4 antibody.

Indeed, the inventors have shown that antibodies directed towards FGFR4 able to inhibit the activity or function of FGFR4 (also called "antagonist anti-FGFR4 antibodies") can have an inhibitory activity on tumor cell proliferation, but only when tumor cells are homozygous for the Gly388 SNP of FGFR4. Further, the inventors have noted that anti-

FGFR4 antibodies have an inhibitory activity only when the receptor FGFR4 with a glycine at position 388 is activated.

Using an animal model (*i.e.* orthotopic liver cancer model in SCID mice via grafting of human cell lines homozygous either for FGFR4-Gly388 or for FGFR4-Arg388), the inventors have shown that liver tumors consisting of human cell lines homozygous for the Gly388 SNP of FGFR4 are efficiently treated with antibodies directed towards FGFR4 (it was observed that the weight of the tumor decreased).

In contrast, it was surprisingly found that treating liver tumors consisting of human cell lines homozygous for the Arg388 SNP of FGFR4 with anti-FGFR4 antibodies is detrimental to the mice. Indeed, the inventors showed that such a treatment stimulates tumor growth.

Consequently, the patients able to respond to an anti-FGFR4 therapy should be homozygous for Gly388 allele of FGFR4 gene.

Therefore, the present invention is particularly useful to distinguish patients suffering from a liver cancer who may benefit from anti-FGFR4 antibody therapies, from those who must not be treated with anti-FGFR4 antibodies.

Definitions

As used herein, "biological sample" can be any sample from the patient, including a blood sample, a serum sample, a plasma sample, or a biopsy of liver tumor, depending on the assay to carry out.

As used herein, "antagonist anti-FGFR4 antibody" refers to any antibody able to specifically bind FGFR4 and prevent, inhibit or reduce its activation.

The activation of FGFR4 encompasses different events involved in its signaling pathway, including autophosphorylation of FGFR4, phosphorylation of different downstream proteins (e.g. Erk1/2, AKT, FRS2, JNK) and induction of cFos and JunB neo-synthesis. Proteins involved in the signaling pathway of FGFR4 are well known in the art, and are for example described by Lin et al., J. Biol. Chem. 2007; French et al., PLoS ONE 2012; Heinzle and *al.*, 2014.

According to the invention, FGFR4 is considered as being activated if the level of activation of FGFR4 in a biological sample is greater than a reference level of activation.

The "reference level of activation of FGFR4" may correspond to the basal level of activation in a biological sample of the same type knowing that FGFR4 activation is only possible when the receptor is expressed at the cell membrane and when ligands are present

(for instance, FGF19 is not produced by normal hepatocytes but is produced in a certain percentage by HCC cells themselves). The reference level of activation of FGFR4 may be reflected through markers (circulating and non-circulating) for which their expression is dependent from FGFR4 activation. The reference level of activation of FGFR4 may also be determined by reference to the level of activation of a given cell or cell line cultured without FGFR4 ligands. The reference level of activation of FGFR4 may be also determined as a single value or a range of values which is determined based on the activation level of FGFR4 measured, for instance, in biological samples of the same type that come from a population of healthy subjects (i.e. subjects who do not suffer from a cancer). This reference level of activation of FGFR4 shows post-prandial fluctuations because FGFR4, in the liver, may be activated by blood FGF19 from intestine neosynthesis following meals, in order to control bile acids pool. Therefore, the reference level of activation and the level of activation of the tissue to be tested both should be determined in the same conditions, i.e. either after meals or before meals. Preferably, the reference level of activation of FGFR4 is determined based on the activation level of FGFR4 measured in liver biopsies (by ELISA dosage or any other methods allowing the determination of FGFR4 phosphorylation level) , or in a roundabout way in liver biopsies or blood samples through circulating and non-circulating markers for which their synthesis is dependent from FGFR4 activation in liver tumor cells, among them alpha-fetoprotein (AFP) which is only detected in blood samples from suffering from HCC, amphiregulin, FOS, EGR1 etc., or by the detection of FGF19 synthesis in tumor cells or in cells from the tumor micro-environment, e.g. endothelial cells, (FGF19 is not expressed by non-pathological liver) associated with the presence of FGFR4 at the cell membrane (increased expression at the cell membrane is observed in HCC compared to normal hepatocytes).

Preferably, a greater level of activation is considered to be statistically significant if the activation level of FGFR4 in the biological sample of the patient is increased 2, 3, 4, 5, 6, 7, 8, 9 or 10 fold, or is increased by at least 25%, and by order of preference at least 30%, 40%, 50%, 60%, 70% or 75% compared with the reference level of activation.

The techniques cited above are described as appropriate methods for measuring activity of FGFR4, but the scope of the invention is not limited by these examples. Any other method known to the skilled person in the art to detect FGFR4 activation can be used.

As used herein, the term "liver cancer" includes hepatocellular carcinoma (abbreviated as "HCC"), cholangiocarcinoma and hepatoblastoma. In particular, liver cancer refers to HCC, a disease which occurs in a subject as a consequence of alcoholism, cirrhosis, infection by hepatotropic viruses (for instance infection by hepatitis C virus or hepatitis B virus), exposure to carcinogen or toxic substance as aflatoxin B.

As used herein, the terms "fibroblast growth factor receptor 4" and "FGFR4" are used interchangeably and refer to all of the naturally-occurring variants (e.g. NCBI accession number NP_998812.1; SEQ ID NO: 3), including allelic variants (such as single nucleotide polymorphisms), of FGFR4 protein. In the present application, cited FGFR4 amino acid positions refer to positions in FGFR4 protein SEQ ID NO: 3.

The terms "homozygous" and "homozygosity", when referring to SNP in codon 388 of FGFR4, means that the same genetic variation, here codon 388 GGG or AGG, is present on both alleles of FGFR4 gene of the homologous chromosomes.

The expression "heterozygous" and "heterozygosity", when referring to SNP in codon 388 of FGFR4, means that a genetic variation, e.g. codon 388 GGG, is present on one allele, and that the other genetic variation, e.g. codon AGG, is present on the other allele of FGFR4 gene of the homologous chromosomes.

As used herein, the terms "subject" and "patient" denote a human or non-human mammal, such as a rodent, a feline, a canine, or a primate. Preferably, the subject and the subject are a human being. However, the veterinary use of the present invention is also contemplated.

The expression "effective amount" is intended to mean an amount sufficient to treat the cancer. It will be appreciated that this amount will vary with the effectiveness of therapeutic agent(s) employed, with the nature of any carrier used, with the seriousness of the disease and the age of the patient. The determination of appropriate amounts for any given composition is within the skill in the art, through standard series of tests designed to assess appropriate therapeutic levels.

By "patient in need" is meant an individual suffering from a cancer, or an individual that is in remission after having suffered from cancer.

The term "treating" is meant to encompass both therapeutic and prophylactic methods, *i.e.* a method aiming at curing, improving the condition and/or extending the lifespan of an individual suffering from the cancer. It also refers to methods aiming at preventing the appearance or the spreading of metastases, as well as methods aiming at preventing a relapse.

The term "antibody" refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that immunospecifically binds an antigen. As such, the term antibody encompasses not only whole antibody molecules, but also antibody fragments as well as variants (including derivatives) of antibodies and antibody fragments.

In particular, the antibody according to the invention may correspond to a polyclonal antibody, a monoclonal antibody (e.g. a chimeric, humanized or human antibody), a fragment of a polyclonal or monoclonal antibody or a diabody.

5 In one embodiment of the invention, the antagonist anti-FGFR4 antagonist is a polyclonal antibody.

A “polyclonal antibody” is an antibody which has been produced from a mixture of antibodies originating from several B lymphocyte clones and which recognize a series of different epitopes.

10 In one advantageous embodiment, the antagonist anti-FGFR4 antagonist is a monoclonal antibody.

A “monoclonal antibody” is an antibody obtained from a substantially homogeneous population of antibodies derived from a single type of B lymphocyte, clonally amplified (for instance a single clone of B cells or hybridoma). The antibodies making up this population are identical except for possible naturally occurring mutations that may be present in minor amounts. These antibodies are directed against a single epitope and are therefore highly specific.

The term “epitope” refers to the site of the antigen to which the antibody binds. If the antigen is a polymer, such as a protein or a polysaccharide, the epitope may be made up of contiguous or noncontiguous residues.

20 In natural antibodies, two heavy chains are linked to each other by disulfide bonds and each heavy chain is linked to a light chain by a disulfide bond. Each chain contains distinct sequence domains. The light chain includes two domains, a variable domain (V_L) and a constant domain (C_L). The heavy chain includes four domains, a variable domain (V_H) and three constant domains (C_{H1} , C_{H2} and C_{H3} , collectively referred to as C_H). The variable regions of both light (V_L) and heavy (V_H) chains determine binding recognition and specificity to the antigen.

The specificity of the antibody resides in the structural complementarity between the antibody combining site and the antigenic determinant. Antibody combining sites are made up of residues that are primarily from the hypervariable or complementarity determining regions (CDRs) as defined by Kabat and al. (Kabat and al., Sequences of proteins of immunological interest, 5th Ed., U.S. Department of Health and Human Services, NIH, 1991, and later editions). They refer to amino acid sequences which, together, define the binding affinity and specificity of the natural Fv region of a native immunoglobulin binding site for the antigen or the epitope that it recognizes. The light and heavy chains of an immunoglobulin each have three CDRs, designated L-CDR1, L-CDR2, L-CDR3 and H-CDR1, H-CDR2, H-

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CDR3, respectively. Therefore, an antigen-binding site includes six CDRs, comprising the CDR set from each of a heavy and a light chain V region.

The most conserved regions of the variable domain are called FR (for “framework”) regions or sequences.

5 “Framework regions” (FRs) refer to amino acid sequences interposed between CDRs, i.e. to those portions of immunoglobulin light and heavy chain variable regions that are relatively conserved among different immunoglobulins in a single species, as defined by Kabat *et al.*, 1991 (Kabat *et al.*, 1991, Sequences of Proteins Of Immunological Interest, National Institute of Health, Bethesda, Md). As used herein, a “human framework region” is a
10 framework region that is substantially identical (about 85%, or more, in particular, 90%, 95% or 100%) to the framework region of naturally occurring human antibody.

The term “VH” refers to the variable regions of an immunoglobulin heavy chain of an antibody, including the heavy chains of an Fv, scFv, dsFv, Fab, Fab’ or F(ab)’ fragment.

15 The term “VL” refers to the variable regions of an immunoglobulin light chain of an antibody, including the light chains of an Fv, scFv, dsFv, Fab, Fab’ or F(ab)’ fragment.

“Antibody fragments” comprise a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fv, Fab, F(ab’)₂, Fab’, Fd, dAb, dsFv, scFv, sc(Fv)₂, CDRs, diabodies and multi-specific antibodies formed from antibodies fragments.

20 The term “Fab” denotes an antibody monovalent fragment having a molecular weight of about 50,000 and antigen binding activity, and consisting of the V_L, V_H, C_L and C_H1 domains.

The Fv fragment is the N-terminal part of the Fab fragment and consists of the variable portions of one light chain and one heavy chain.

25 The term “F(ab’)₂” refers to an antibody bivalent fragment having a molecular weight of about 100,000 and antigen binding activity, which comprises two Fab fragments linked by a disulfide bridge at the hinge region.

The term “Fab” refers to an antibody fragment having a molecular weight of about 50,000 and antigen binding activity, which is obtained by cutting a disulfide bond of the hinge region of the F(ab’)₂ fragment.

30 The term “Fd” refers to an antibody fragment consisting of the V_H and C_H1 domains.

The term “dAb” (Ward *et al.*, 1989 Nature 341:544-546) refers to a single variable domain antibody, i.e. an antibody fragment which consists of a V_H or V_L domain.

A single chain Fv ("scFv") polypeptide is a covalently linked $V_H::V_L$ heterodimer which is usually expressed from a gene fusion including V_H and V_L encoding genes linked by a peptide-encoding linker. "dsFv" is a $V_H::V_L$ heterodimer stabilised by a disulfide bond. Divalent and multivalent antibody fragments can form either spontaneously by association of monovalent scFvs, or can be generated by coupling monovalent scFvs by a peptide linker, such as divalent sc(Fv)₂.

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a V_H domain connected to a V_L domain in the same polypeptide chain (V_H - V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementarity domains of another chain and create two antigen-binding sites.

The term "chimeric antibody" refers to an antibody in which the constant region, or a portion thereof, is altered, replaced or exchanged, such that the variable region is linked to a constant region of a different species, or belongs to another antibody class or subclass. The term "chimeric antibody" also refers to an antibody in which the variable region, or a portion thereof, is altered, replaced or exchanged, such that the constant region is linked to a variable region of a different species, or belongs to another antibody class or subclass.

The methods of producing chimeric antibodies are known to those skilled in the art. See, for example, Morrison, 1985, Science, 229:1202; Oi and al., 1986, Bio Techniques, 4:214; Gillies and al., 1989, J. Immunol. Methods, 125:191-202; U.S. Pat. Nos. 5,807,715; 4,816,567; and 4,816,397.

These chimeric versions of the antibody may comprise the fusion of the V_L and V_H variable regions to the C_kappa and the CH (IgG1) constant domains of human origin in order to generate a chimeric monoclonal antibody.

The CH (IgG1) domain can also be modified by point mutations in order to increase the affinity of the Fc fragment for the $Fc\gamma RIIIa$ receptor and thereby to increase the effector functions of the antibody (Lazar and al., 2006, Proc. Natl. Acad. Sci. USA 103: 4005-4010; Stavenhagen and al., 2007, Cancer Res. 67: 8882-8890).

The present invention includes the humanized versions of the antibodies.

The term "humanized antibody" refers to an antibody which contains mainly human immunoglobulin sequences. This term generally refers to a non-human immunoglobulin which has been modified by incorporation of human sequences or of residues found in human sequences.

Typically, a humanized antibody is a chimeric, genetically engineered, antibody in which the CDRs from a mouse antibody ("donor antibody") are grafted onto a human antibody ("acceptor antibody"). Thus, a humanized antibody is an antibody having CDRs from a donor antibody and variable region framework and constant regions from a human antibody. The use of antibody components derived from humanized monoclonal antibodies obviates potential problems associated with the immunogenicity of murine constant regions.

In general, humanized antibodies comprise one or typically two variable domains in which all or part of the CDR regions correspond to parts derived from the non-human parent sequence and in which all or part of the FR regions are those derived from a human immunoglobulin sequence. The humanized antibody can then comprise at least one portion of an immunoglobulin constant region (Fc), in particular that of the human immunoglobulin template chosen.

The goal is thus to have an antibody that is minimally immunogenic in a human. Thus it is possible that one or two amino acids in one or more CDRs can be modified by one that is less immunogenic to a human host, without substantially reducing the specific binding function of the antibody to FGF-R4. Similarly, the residues of the framework regions may not be human, and it is possible for them not to be modified since they do not contribute to the immunogenic potential of the antibody.

Several methods of humanization known to those skilled in the art exist for modifying a non-human parent antibody so as to give an antibody that is less immunogenic to humans. An overall sequence identity with a human antibody is not necessarily required. This is because the overall sequence identity is not necessarily a predictive indicator of reduced immunogenicity, and the modification of a limited number of residues can result in humanized antibodies having a greatly reduced immunogenic potential in humans (Molecular Immunology (2007) 44, 1986-1998).

Various methods are, for example, CDR grafting (EP 0 239 400; WO 91/09967; and U.S. Pat. Nos. 5,530,101 and 5,585,089), resurfacing (EP 0 592 106; EP 0 519 596; Padlan, 1991, Molec Imm 28(4/5):489-498; Studnicka and al., 1994, Prot Eng 7(6):805-814; and Roguska and al., 1994, PNAS 91:969-973) or else chain shuffling (U.S. Pat. No. 5,565,332).

In a particular embodiment of the invention, humanized antibodies of murine antibody 40-12 are obtained as follows:

The light and heavy chains most similar to the corresponding chains of the anti-FGFR4 murine antibody 40-12 are identified by comparison with the Protein Data Bank (H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne. Nucleic Acids Research, 2000, 28:235-242). The sequence alignment uses the

BLAST algorithm (J Mol Biol. 1990 Oct 215:403-410). These are tridimensional structures corresponding to the PDB codes 1NDM & 1ETZ respectively used to build up the homology models of the variable domain light and heavy chains. These tridimensional models are subsequently energy minimized using the standard procedure implemented in the MOE software (Molecular Operating Environment, Chemical Computing Group, Quebec, Canada). A molecular dynamic (MD) simulation of these minimized tridimensional models of the antibody is subsequently performed with the Amber software (D.A. Case, T.E. Cheatham, III, T. Darden, H. Gohlke, R. Luo, K.M. Merz, Jr., A. Onufriev, C. Simmerling, B. Wang and R. Woods. J. Computat. Chem. 2005, 26:1668-1688). This simulation is done with harmonic constraints applied to the protein backbone atoms at a temperature of 500 K for a period of 1.1 nanoseconds in a generalized Born implicit solvent (Gallicchio & Levy, J Comput Chem 2004, 25:479-499). Ten diverse conformations are thus extracted from this first simulation, one tridimensional conformation every one hundred picoseconds, during the last nanosecond of the simulation. These ten diverse conformations are then each subjected to a molecular dynamic simulation, without constraints on the protein backbone, at a temperature of 27°C for 2.3 nanoseconds in a generalized Born implicit solvent. The bonds involving a hydrogen atom are constrained using the SHAKE algorithm (Barth. and al., J Comp Chem, 1995, 16:1192-1209), the time step is 1 femtosecond, and the simulation was run based on the Langevin equation at constant volume and a constant temperature of 27°C. For each of the ten molecular dynamic simulations, the last two thousand conformations, extracted at a frequency of one every picosecond, are then used to quantify, for each amino acid of the antibody to be humanized, the deviation of the atomic positions with respect to an average, or medoid, conformation of the amino acid. The Scientific Vector Language (SVL) of the MOE software is used to code all of the analysis described below. The medoid conformation of the amino acid is the conformation derived from the molecular dynamic which is the closest to the average conformation calculated from the position of the atoms of all the conformations of the amino acid. The distance used for detecting the medoid conformation is the route mean square (RMSD) of the scalar distances between the atoms of two conformations of the amino acid. Similarly, the deviation of the positions of the atoms of one conformation of an amino acid compared with the medoid conformation is quantified by calculating the RMSD of the scalar distances between the atoms of the amino acid of one conformation of the simulation and the same atoms of the medoid conformation. Subsequently, by comparing the RMSD of the positions of the atoms of a given amino acid (i), averaged over all the ten molecular dynamic simulations (Fi), with the RMSD of the positions of all the amino acids of the antibody, averaged over all the ten molecular dynamic simulations (Fm), it is decided whether the amino acid is flexible enough to be considered able to potentially interact with the T-cell receptors and trigger activation of the immune

system. An amino acid i is considered flexible if its flexibility score Z_i , defined as $Z_i = (F_i - F_m)/F_m$, is above 0.15. 45 amino acids are thus identified as flexible in the variable domain of the antibody, with the exclusion of the antigen complementarity determining region (CDR) and of its immediate vicinity. The immediate vicinity of the CDR is defined as any amino acid with an alpha carbon at a distance of 5 angströms (Å) or less to an alpha carbon of the CDR.

The motions of the 60 most flexible amino acids of the antibody, during the 20 nanoseconds (10x2ns) of simulation, are then compared to the motions of the corresponding amino acids of 49 homology models of human antibody germ lines, for each of which ten molecular dynamic simulations (10x2 ns) have been run using the same protocol. The 60 most flexible amino acids exclude the antigen complementarity determining region (CDR) and its immediate vicinity. The 49 human antibody germ line models were built by systematically combining the 7 most frequent human light chains (vk1, vk2, vk3, vk4, vlamba1, vlamba2, vlamba3) and the 7 most frequent human heavy chains (vh1a, vh1b, vh2, vh3, vh4, vh5, vh6) (Nucleic Acids Research, 2005, Vol. 33, Database issue D593-D597).

The similarity of the antibody to be humanized to the 49 human germ line models is quantified by sampling the positions of specific atoms of the 60 flexible amino acids of an antibody, over the course of the ten molecular dynamic simulations, by means of a unique tridimensional cubic grid which has a 1Å resolution. This is referred to as quadridimensional similarity. The tridimensional grid used is made of 445 740 points and is initialized using the tridimensional structure of the human antibody corresponding to the PDB code 8FAB. The 8FAB structure is also used to position all the conformations of an antibody to be sampled in the tridimensional grid. For this purpose, the medoid conformation of the molecular dynamic of the antibody is superposed onto the 8FAB structure. This superposition consists of aligning the moments of inertia of the two conformations, followed by the optimization of the scalar distances between the alpha carbon atoms of both conformations. All the remaining conformations of the molecular dynamic of the antibody are superposed onto the medoid conformation using the same method.

Two types of sampling are performed, which result in two similarities (electrostatic similarity and lipophilic similarity), for a pair of antibodies being compared. These two similarities are then added to obtain the total similarity. The first sampling, the electrostatic sampling, considers all atoms of the amino acid side chain. The value in one point, x , of the grid is obtained by applying, to the atoms of the amino acid side chain, a tridimensional Gaussian function $f(x)$ weighted with the atomic partial charge as described in the Amber99 force field (Cieplak, J., and al.; J. Comp. Chem. 2001, 22:1048-1057).

The $f(x)$ function is applied on the 3 Cartesian coordinate axes and corresponds to the following formula: $f(x) = \left(s\sqrt{2\pi}\right)^{-3} \times \exp\left(\frac{-(x-u)^2}{2s^2}\right)$, with x and u being, respectively, the Cartesian coordinates of a grid point x and of a sampled atom, and $s = r/1.6$ (r = covalence radius of the atom). The sampling is repeated for all conformations of the amino acid and the obtained results are averaged at all points x of the tridimensional grid. The second sampling, the lipophilic sampling, considers only the lipophilic atoms of the amino acid side chain. The value at one point, x , of the grid is calculated with the same Gaussian function $f(x)$ without weighting. As a result, the two ensembles of conformations from the molecular dynamic simulations, of the two antibodies being compared, are sampled by the same tridimensional grid.

The electrostatic similarity (sim-elec) between antibody a and antibody b is measured with the following formula:

$$\text{sim} - \text{elec} = \frac{\sum_{i=1}^{445740} \left(\left| x_i^a + x_i^b \right| - \left| x_i^a - x_i^b \right| \right)}{\sum_{i=1}^{445740} \left(\left| x_i^a + x_i^b \right| \right)}.$$

The lipophilic similarity is calculated with the same formula applied to the data generated by the lipophilic sampling previously described.

The human germ line model vlambda2-vh2 thus displays the highest quadridimensional similarity (total similarity = 58%) of these 60 flexible amino acids with respect to the flexible amino acids of the murine antibody 40-12. The human germ line model vlambda2-vh2 has thus been used to humanize the antibody to be humanized, while focusing on the 45 flexible amino acids. In order to determine the mutations to be made, the tridimensional structure of the model of the murine antibody 40-12 is superposed on that of the model derived from the germ lines showing the highest similarity, with the positions of the alpha carbons of the amino acids being optimized. The amino acids identified as flexible are mutated with the corresponding amino acids in the sequence of the model showing the highest similarity.

Antibodies 8, 31, 33 and 36 can also be humanized following a similar procedure.

The unwanted sequence motifs considered are the following: Aspartate-Proline (peptide bond labile in acidic medium), Asparagine-X-Serine/Threonine (glycosylation site, X=any amino acid except Proline), Aspartate-Glycine/Serine/Threonine (potential formation of succinimide/isoaspartate in the flexible zones), Asparagine-Glycine/Histidine/Serine/Alanine/Cysteine (exposed deamidation sites), Methionine (oxidation in the exposed zones). The humanized sequences thus obtained are finally compared, by means of the

BLAST sequence comparison algorithm, with the sequences of the IEDB database (<http://www.immuneepitope.org/>; The immune epitope database and analysis resource: from vision to blueprint. PLoS Biol. 2005 Mar;3(3):e91) so as to be sure that the sequences do not contain any epitopes known to be recognized by B and T lymphocytes. If the sequence contains residues which have unwanted sequences, they are then also modified. If the composite sequence contains a known epitope listed in the IEDB, another germ line structure template showing a high similarity is then used as model.

Antibodies according to the invention may be produced by any technique known in the art, such as, without limitation, any chemical, biological, genetic or enzymatic technique, either alone or in combination. The antibodies of this invention can be obtained by producing and culturing hybridomas or by phage display according to methods known to those skilled in the art (McCafferty J. and al, 1990; Hoogenboom, HR and al, 2005). Other technologies are available for the preparation of human antibodies, such as the XenoMouse technology described in US patent 5,939,598.

Antagonist anti-FGFR4 antibodies of the invention include those described in international application WO2005/037235, international application WO2010/004204), international applications WO2008/052796 and WO2008/052798 (in particular monoclonal antibody 10F10 or an antibody which comprises six CDRs identical to monoclonal antibody 10F10), international application WO2010/026291 (in particular monoclonal antibody F90-10C5 or an antibody which comprises six CDRs identical to monoclonal antibody F90-10C5 CDRs), antibody LD1 described by Dorothy M. French *et al.* (PLoS ONE, May 2012, 7(5); e36713) or an antibody which comprises six CDRs identical to monoclonal antibody LD1, the anti-FGFR-4 antibody disclosed by Xoma Ltd (see on the website <http://www.xoma.com/content/pipeline/oncology.htm>).

Preferably, the antagonist anti-FGFR4 antibody of the invention binds to an epitope belonging to the D2-D3 domain of the FGFR4 receptor. More preferably, the antibody binds an epitope included in the domain comprising amino acids 144 to 365 of the FGFR4 receptor. More preferably, the antibody binds an epitope included in the D2 domain of the FGFR4 receptor, this epitope corresponding to amino acids 145 to 242 described in the sequence SEQ ID No. 4.

Preferably, the antagonist anti-FGFR4 antibody induces inhibition of AKT/p38 cell pathways and/or Erk1/2 cell pathways, and/or FGFR4-controlled cell signaling pathways.

Preferably, the antagonist anti-FGFR4 antibody induces inhibition of tumor cell proliferation and/or angiogenesis.

Preferably, the antagonist anti-FGFR4 antibody comprises at least one CDR of sequence SEQ ID No. 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33 or 34 or at least one CDR the sequence of which differs by one or two amino acids from sequences SEQ ID No. 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33 or 34, provided that the antibody keeps its binding specificity and its antagonist effect on FGFR4.

Preferably, the antagonist anti-FGFR4 antibody according to the invention comprise at least one heavy chain and at least one light chain, said heavy chain comprising three CDR sequences having amino acid sequences chosen from the group consisting of SEQ ID No. 5, 6 and 7 or 11, 12 and 13, 17, 18 and 19, or 23, 24 and 25, or 29, 30 and 31, said light chain comprising three CDR sequences having amino acid sequences chosen from the group consisting of SEQ ID No. 8, 9 and 10 or 14, 15 and 16, or 20, 21 and 22, or 26, 27 and 28, or 32, 33 and 34.

Preferably, the heavy chain variable region of the antagonist anti-FGFR4 antibody (i) comprises, or consists of, a sequence chosen from the group consisting of sequence SEQ ID No. 40, 56, 60, 64 or 68, or (ii) alternatively comprises, or consists of, a sequence that has at least 80%, 90%, 95%, 96%, 97%, 98% or 99% amino acid sequence identity to a full-length polypeptide sequence chosen from the group consisting of SEQ ID No. 40, 56, 60, 64 and 68.

Preferably, the light chain variable regions of the antagonist anti-FGFR4 antibody (i) comprises, or consists of, a sequence chosen from the group consisting of SEQ ID No. 42, 54, 58, 62 or 66, or (ii) alternatively comprises, or consists of, a sequence that has at least 80%, 90%, 95%, 96%, 97%, 98% or 99% amino acid sequence identity to a full-length polypeptide sequence chosen from the group consisting of SEQ ID No. 42, 54, 58, 62 and 66.

Preferably, the antagonist anti-FGFR4 antibody comprises a heavy chain variable region encoded by a nucleotide sequence comprising, or consisting of, a having at least 80%, 90%, 95%, 96%, 97%, 98% or 99% of sequence identity with a sequence chosen from the group consisting of SEQ ID No. 39, 55, 59, 63 and 67.

Preferably, the antagonist anti-FGFR4 antibody comprises a light chain variable region encoded by a nucleotide sequence comprising, or consisting of, a sequence having at least 80%, 90%, 95%, 96%, 97%, 98% or 99% of sequence identity with a sequence chosen from the group consisting of SEQ ID No. 41, 53, 57, 61 or 65.

Preferably, the antagonist anti-FGFR4 antibody comprises a heavy chain encoded by a nucleotide sequence having at least 80%, 90%, 95%, 96%, 97%, 98% or 99% of sequence identity with SEQ ID No. 35.

5 Preferably, the antagonist anti-FGFR4 antibody comprises a heavy chain of polypeptide sequence having at least 80%, 90%, 95%, 96%, 97%, 98% or 99% of sequence identity with SEQ ID No. 36.

Preferably, the antagonist anti-FGFR4 antibody comprises a light chain encoded by a nucleotide sequence having at least 80%, 90%, 95%, 96%, 97%, 98% or 99% of sequence identity with SEQ ID No. 37.

10 Preferably, the antagonist anti-FGFR4 antibody comprises a light chain of polypeptide sequence having at least 80%, 90%, 95%, 96%, 97%, 98% or 99% of sequence identity with SEQ ID No. 38.

More preferably, the antagonist anti-FGFR4 antibody comprises the sequences encoded by nucleotide sequences SEQ ID No. 35 and 37.

15 More preferably, the antagonist anti-FGFR4 antibody comprises a heavy chain comprising the sequence SEQ ID No. 36 and a light chain comprising the sequence SEQ ID No. 38.

Preferably, the antagonist anti-FGFR4 antibody is a human antibody of which the heavy chain variable regions are encoded by polynucleotide sequences which comprise a
20 nucleotide sequence having at least 80%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID No. 55, 59, 63 or 67.

Preferably, the antagonist anti-FGFR4 antibody is a human antibody of which the light chain variable regions are encoded by polynucleotide sequences which comprise a
25 nucleotide sequence having at least 80%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID No. 53, 57, 61 or 65.

Preferably, the antagonist anti-FGFR4 antibody is a human antibody comprising a heavy chain the variable region of which has at least 80%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID No. 56, 60, 64 or 68.

30 Preferably, the antagonist anti-FGFR4 antibody is a human antibody comprising a light chain the variable region of which has at least 80%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID No. 54, 58, 62 or 66.

More preferably, the antagonist anti-FGFR4 antibody comprises a sequence at least 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID No. 54 and/or a sequence at least 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID No. 56.

More preferably, the antagonist anti-FGFR4 antibody comprises a sequence at least 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID No. 58 and/or a sequence at least 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID No. 60.

5 More preferably, the antagonist anti-FGFR4 antibody comprises a sequence at least 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID No. 62 and/or a sequence at least 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID No. 64.

More preferably, the antagonist anti-FGFR4 antibody comprises a sequence at least 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID No. 66 and/or a sequence at least 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID No. 68.

10 The antibody composed of a heavy chain of sequence SEQ ID No. 36 and of a light sequence SEQ ID No. 38 is referred to "antibody 40-12" in the present application.

The antibody composed of a heavy chain of sequence SEQ ID No. 56 and of a light sequence SEQ ID No. 54 is referred to "clone 8" or "Antibody 8" (*Ab* 8) in the present application.

15 The antibody composed of a heavy chain of sequence SEQ ID No. 60 and of a light sequence SEQ ID No. 58 is referred to "clone 31" or "Antibody 31" (*Ab* 31) in the present application.

The antibody composed of a heavy chain of sequence SEQ ID No. 64 and of a light sequence SEQ ID No. 62 is referred to "clone 33" or "Antibody 33" (*Ab* 33) in the present application.

20 The antibody composed of a heavy chain of sequence SEQ ID No. 68 and of a light sequence SEQ ID No. 66 is referred to "clone 36" or "Antibody 36" (*Ab* 36) in the present application.

Even more preferably, the antagonist anti-FGFR4 antibody of the invention comprises:

- 25 i) six CDRs having the amino acid sequences SEQ ID Nos: 5, 6, 7, 8, 9, 10; or
- ii) six CDRs having the amino acid sequences SEQ ID Nos: 11, 12, 13, 14, 15, 16; or
- iii) six CDRs having the amino acid sequences SEQ ID Nos: 17, 18, 19, 20, 21, 22; or
- iv) six CDRs having the amino acid sequences SEQ ID Nos: 23, 24, 25, 26, 27, 28; or
- v) six CDRs having the amino acid sequences SEQ ID Nos: 29, 30, 31, 32, 33 or 34; or
- 30 vi) six CDRs the sequences of which differ from their corresponding respective sequences mentioned at point (i), (ii), (iii), (iv) or (v) respectively, at most by one or two

amino acids, provided that the antibody keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4.

Even more preferably, the antagonist anti-FGFR4 antibody comprises a heavy chain variable region and a light chain variable region encoded by nucleotide sequences chosen from the group consisting of:

- (i) SEQ ID Nos. 39 and 41;
- (ii) SEQ ID Nos. 53 and 55;
- (iii) SEQ ID Nos. 57 and 59;
- (iv) SEQ ID Nos. 61 and 63, and
- (v) SEQ ID Nos. 65 and 67;

(vi) a heavy chain variable region and a light chain variable region encoded by nucleotide sequences at least 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to (i), (ii), (iii), (iv) or (v), provided that the antibody keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4.

Even more preferably, the antagonist anti-FGFR4 antibody comprises polypeptide sequences chosen from the group consisting of:

- (i) SEQ ID No. 36 and SEQ ID No. 38; or
- (ii) SEQ ID Nos. 40 and 42; or
- (iii) SEQ ID No. 54 and SEQ ID No. 56; or
- (iv) SEQ ID No. 58 and SEQ ID No. 60; or
- (v) SEQ ID No. 62 and SEQ ID No. 64; or
- (vi) SEQ ID No. 66 and SEQ ID No. 68;

(vii) two polypeptide sequences at least 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to polypeptides of (i), (ii), (iii), (iv), (v) or (vi), provided that the antibody keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4.

Most preferably, the antagonist anti-FGFR4 antibody is chosen from the group consisting of antibody 40-12, antibody 8, antibody 31, antibody 33, antibody 36 and antibody LD1, or a humanized antagonist anti-FGFR4 antibody thereof.

In an implementation of the invention, the antagonist anti-FGFR4 antibody is a humanized antagonist anti-FGFR4 antibody.

Preferably, the humanized antagonist anti-FGFR4 antibody comprises a light chain the variable region of which is encoded by a nucleotide sequence consisting of SEQ ID No. 43 or
5 SEQ ID No. 45, or alternatively which is encoded by a nucleotide sequence which has at least 80%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID No. 43 or SEQ ID No. 45.

Preferably, the humanized antagonist anti-FGFR4 antibody comprises a light chain the variable region of which is SEQ ID No. 44 or SEQ ID No. 46, or alternatively a sequence
10 which has at least 80%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID No. 44 or SEQ ID No. 46.

Preferably, the humanized antagonist anti-FGFR4 antibody comprises a heavy chain the variable region of which is encoded by a nucleotide sequence chosen from the group consisting of SEQ ID No. 47, SEQ ID No. 49 and SEQ ID No. 51, or alternatively which is
15 encoded by a nucleotide which has at least 80%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity with a sequence chosen from the group consisting of SEQ ID No. 47, SEQ ID No. 49 and SEQ ID No. 51.

Preferably, the humanized antagonist anti-FGFR4 antibody comprises a heavy chain the variable region of which is chosen from the group consisting of SEQ ID No. 48, SEQ ID
20 No. 50 and SEQ ID No. 52, or alternatively a sequence which has at least 80%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity with a sequence chosen from the group consisting of SEQ ID No. 48, SEQ ID No. 50 and SEQ ID No. 52.

More preferably, the humanized antagonist anti-FGFR4 antibody comprises a light chain the variable region of which is SEQ ID No. 44 or SEQ ID No. 46 and a heavy chain the variable region of which is chosen from the group consisting of SEQ ID No. 48, SEQ ID No.
25 50 and SEQ ID No. 52.

In a particular embodiment, the antagonist anti-FGFR4 antibody comprises:

- a CDRH1 comprising or consisting of sequence SEQ ID NO: 71, SEQ ID NO: 5,
30 SEQ ID NO: 95 or a sequence differing from one of these sequences by one or two amino acid substitutions,
- a CDRH2 comprising or consisting of a sequence selected from the group consisting of:
 - a) SEQ ID NO: 6,

- 5
- b) SEQ ID NO: 96,
 - c) a sequence differing from a) or b) by one or two amino acid substitutions, optionally provided that none of said one or two amino acid substitution(s) is located at position 11 of SEQ ID NO: 6,
 - d) SEQ ID NO: 6 with one amino acid substitution at position 11 and
 - e) a sequence differing from d) by one or two additional amino acid substitution(s) provided that none of said one or two additional amino acid substitution(s) is located at position 11 of SEQ ID NO: 6,
- 10
- a CDRH3 comprising or consisting of a sequence selected from the group consisting of:
 - a) SEQ ID NO: 73,
 - 15 b) SEQ ID NO: 7,
 - and
 - c) a sequence differing from a) or b) by one or two amino acid substitutions,
 - a CDRL1 comprising or consisting of a sequence selected from the group consisting of:

20

 - a) SEQ ID NO: 8,
 - b) SEQ ID NO: 97,
 - c) a sequence differing from a) or b) by one or two amino acid substitutions, optionally provided that none of said one or two amino acid substitution(s) is located at position 8 of SEQ ID NO: 8 or at position 5 of SEQ ID NO: 97,
 - 25 d) SEQ ID NO: 8 with one amino acid substitution at position 8,
 - e) a sequence differing from d) by one or two additional amino acid substitution(s) provided that none of said one or two additional amino acid substitution(s) is located at position 8 of SEQ ID NO: 8,
 - 30 f) SEQ ID NO: 97 with one amino acid substitution at position 5, and
 - g) a sequence differing from f) by one or two additional amino acid substitution(s) provided that none of said one or two additional
- 35

amino acid substitution(s) is located at position 5 of SEQ ID NO: 97,

- a CDRL2 comprising or consisting of a sequence selected from the group consisting of:

- 5 a) SEQ ID NO: 9,
- b) SEQ ID NO: 98,
- c) a sequence differing from a) or b) by one or two amino acid substitutions, optionally provided that none of said one or two amino acid substitution(s) is located at position 4 of
10 SEQ ID NO: 9,
- d) SEQ ID NO: 9 with one amino acid substitution at position 4
 and
- e) a sequence differing from d) by one or two additional amino acid substitution(s) provided that none of said one or two
15 additional amino acid substitution(s) is located at position 4 of
 SEQ ID NO: 9,

and

- a CDRL3 comprising or consisting of a sequence selected from the group consisting of:

- 20 a) SEQ ID NO: 10,
- b) a sequence differing from a) by one or two amino acid substitutions, optionally provided that none of said one or two amino acid substitution(s) is located at position 4 of SEQ ID NO: 10,
- 25 c) SEQ ID NO: 10 with one amino acid substitution at position 4
 and
- d) a sequence differing from c) by one or two additional amino acid substitution(s) provided that none of said one or two additional amino acid substitution(s) is located at position 4 of
30 SEQ ID NO: 10;

provided that said antibody comprises at least one CDR chosen from the group consisting of:

- a) a CDRH2 comprising or consisting of SEQ ID NO: 6 with one amino acid substitution at position 11;

- 5 b) a CDRH2, the sequence of which differs from a) by one or two additional amino acid substitution(s) provided that none of said one or two additional amino acid substitution(s) is located at position 11 of SEQ ID NO: 6;
- c) a CDRL1 comprising or consisting of sequence SEQ ID NO: 8 with one amino acid substitution at position 8;
- 10 d) a CDRL1, the sequence of which differs from c) by one or two additional amino acid substitution(s) provided that none of said one or two additional amino acid substitution(s) is located at position 8 of SEQ ID NO: 8;
- e) a CDRL1 comprising or consisting of sequence SEQ ID NO: 97 with one amino acid substitution at position 5;
- 15 f) a CDRL1, the sequence of which differs from e) by one or two additional amino acid substitution(s) provided that none of said one or two additional amino acid substitution(s) is located at position 5 of SEQ ID NO: 97;
- g) a CDRL2 comprising or consisting of sequence SEQ ID NO: 9 with one amino acid substitution at position 4;
- 20 h) a CDRL2, the sequence of which differs from g) by one or two additional amino acid substitution(s) provided that none of said one or two additional amino acid substitution(s) is located at position 4 of SEQ ID NO: 9;
- i) a CDRL3 comprising or consisting of sequence SEQ ID NO: 10 with one amino acid substitution at position 4; and
- 25 j) a CDRL3, the sequence of which differs from i) by one or two additional amino acid substitution(s) provided that none of said one or two additional amino acid substitution(s) is located at position 4 of SEQ ID NO: 10.

30 Preferably, the antagonist anti-FGFR4 antibody of the particular embodiment as defined above comprises at least one CDR, at least two CDRs or at least three CDRs, chosen from the group consisting of:

- a) a CDRH2 comprising or consisting of SEQ ID NO: 6 with one amino acid substitution at position 11;
- 35 b) a CDRH2, the sequence of which differs from a) by one or two additional amino acid substitution(s) provided that none of said one

or two additional amino acid substitution(s) is located at position 11 of SEQ ID NO: 6;

c) a CDRL1 comprising or consisting of sequence SEQ ID NO: 8 with one amino acid substitution at position 8;

d) a CDRL1, the sequence of which differs from c) by one or two additional amino acid substitution(s) provided that none of said one or two additional amino acid substitution(s) is located at position 8 of SEQ ID NO: 8;

e) a CDRL1 comprising or consisting of sequence SEQ ID NO: 97 with one amino acid substitution at position 5;

f) a CDRL1, the sequence of which differs from e) by one or two additional amino acid substitution(s) provided that none of said one or two additional amino acid substitution(s) is located at position 5 of SEQ ID NO: 97;

g) a CDRL2 comprising or consisting of sequence SEQ ID NO: 9 with one amino acid substitution at position 4;

h) a CDRL2, the sequence of which differs from g) by one or two additional amino acid substitution(s) provided that none of said one or two additional amino acid substitution(s) is located at position 4 of SEQ ID NO: 9;

i) a CDRL3 comprising or consisting of sequence SEQ ID NO: 10 with one amino acid substitution at position 4; and

j) a CDRL3, the sequence of which differs from i) by one or two additional amino acid substitution(s) provided that none of said one or two additional amino acid substitution(s) is located at position 4 of SEQ ID NO: 10.

Preferably, the antagonist anti-FGFR4 antibody of the particular embodiment as defined above comprises at least one CDR, at least two CDRs or at least three CDRs chosen from the group consisting of:

- a CDRL1 of c), d), e) or f),
- a CDRL2 of g) or h), and
- a CDRL3 of i) or j).

Preferably, the antagonist anti-FGFR4 antibody of the particular embodiment as defined above comprises four CDRs chosen from the above-mentioned group:

- a CDRH2 of a) or b),
- a CDRL1 of c), d), e) or f),
- a CDRL2 of g) or h), and
- a CDRL3 of i) or j).

5

The CDRH2 of the antagonist anti-FGFR4 antibody as defined above may particularly comprise or consist of a sequence differing from SEQ ID NO: 6 by one amino acid substitution at position 11 selected from the group consisting of N11S, N11Q and N11T and, optionally, one or two other amino acid substitutions.

10

The CDRL1 of the antagonist anti-FGFR4 antibody as defined above may particularly comprise or consist of:

- a sequence differing from SEQ ID NO: 8 by one amino acid substitution at position 8 selected from the group consisting of N8Y, N8L, N8H, N8F, N8I, N8V and, optionally, one or two other amino acid substitutions, or
- a sequence differing from SEQ ID NO: 97 by one amino acid substitution at position 5 selected from the group consisting of N5Y, N5L, N5H, N5F, N5I, N5V and, optionally, one or two other amino acid substitutions.

15

The CDRL2 of the antagonist anti-FGFR4 antibody as defined above may particularly comprise or consist of a sequence differing from SEQ ID NO: 9 by one amino acid substitution at position 4 selected from the group consisting of Q4L, Q4I, Q4V and, optionally, one or two other amino acid substitutions.

20

The CDRL3 of the antagonist anti-FGFR4 antibody as defined above may particularly comprise or consist of a sequence differing from SEQ ID NO: 10 by one amino acid substitution at position 4 selected from the group consisting of N4D, N4E and, optionally, one or two other amino acid substitutions.

25

More preferably, the antagonist anti-FGFR4 antibody of the particular embodiment as defined above comprises:

- a CDRH1 comprising or consisting of sequence SEQ ID NO: 71, SEQ ID NO: 5, SEQ ID NO: 95 or a sequence differing from one of these sequences by one or two amino acid substitutions
- a CDRH2 comprising or consisting of sequence SEQ ID NO: 72, SEQ ID NO: 6, SEQ ID NO: 96 or a sequence differing from one or two amino acid substitutions, provided that none of said one or two amino acid substitution(s) is located at position 11 of SEQ ID NO: 72,

30

- a CDRH3 comprising or consisting of sequence SEQ ID NO: 73 or SEQ ID NO: 7, or a sequence differing from one of these sequences by one or two amino acid substitutions,
- 5 - a CDRL1 comprising or consisting of sequence SEQ ID NO: 80, SEQ ID NO: 94 or a sequence differing from one of these sequences by one or two amino acid substitutions provided that none of said one or two amino acid substitution(s) is located at position 8 of SEQ ID NO: 80 or at position 5 of SEQ ID NO: 94,
- 10 - a CDRL2 comprising or consisting of sequence SEQ ID NO: 81 or a sequence differing from one of this sequence by one or two amino acid substitutions, provided that none of said one or two amino acid substitution(s) is located at position 4 of SEQ ID NO: 81, and
- 15 - a CDRL3 comprising or consisting of sequence SEQ ID NO: 82 or a sequence differing from one of this sequence by one or two amino acid substitutions provided that none of said one or two amino acid substitution(s) is located at position 4 of SEQ ID NO: 82.

More preferably, the antagonist anti-FGFR4 antibody of the particular embodiment as defined above comprises:

- 20 - a CDRH1 comprising or consisting of sequence SEQ ID NO: 71, SEQ ID NO: 5 or SEQ ID NO: 95,
- a CDRH2 comprising or consisting of sequence SEQ ID NO: 72,
- a CDRH3 comprising or consisting of sequence SEQ ID NO: 73 or SEQ ID NO: 7,
- 25 - a CDRL1 comprising or consisting of sequence SEQ ID NO: 80 or SEQ ID NO: 94,
- a CDRL2 comprising or consisting of sequence SEQ ID NO: 81, and
- a CDRL3 comprising or consisting of sequence SEQ ID NO: 82.

30 More preferably, the antagonist anti-FGFR4 antibody of the particular embodiment as defined above comprises:

- 35 - a CDRH1 comprising or consisting of sequence SEQ ID NO: 71,
- a CDRH2 comprising or consisting of sequence SEQ ID NO: 72,
- a CDRH3 comprising or consisting of sequence SEQ ID NO: 73,
- a CDRL1 comprising or consisting of sequence SEQ ID NO: 80,
- a CDRL2 comprising or consisting of sequence SEQ ID NO: 81, and

- a CDRL3 comprising or consisting of sequence SEQ ID NO: 82.

More preferably, the antagonist anti-FGFR4 antibody of the particular embodiment as defined above comprises:

- 5 - a CDRH1 comprising or consisting of sequence SEQ ID NO: 5,
- a CDRH2 comprising or consisting of sequence SEQ ID NO: 72,
- a CDRH3 comprising or consisting of sequence SEQ ID NO: 7,
- a CDRL1 comprising or consisting of sequence SEQ ID NO: 80,
- a CDRL2 comprising or consisting of sequence SEQ ID NO: 81, and
- 10 - a CDRL3 comprising or consisting of sequence SEQ ID NO: 82.

More preferably, the antagonist anti-FGFR4 antibody of the particular embodiment as defined above comprises:

- a CDRH1 comprising or consisting of sequence SEQ ID NO: 5,
- 15 - a CDRH2 comprising or consisting of sequence SEQ ID NO: 6,
- a CDRH3 comprising or consisting of sequence SEQ ID NO: 7,
- a CDRL1 comprising or consisting of sequence SEQ ID NO: 80,
- a CDRL2 comprising or consisting of sequence SEQ ID NO: 81, and
- a CDRL3 comprising or consisting of sequence SEQ ID NO: 82.

20

Preferably, the heavy chain variable region of the antagonist anti-FGFR4 antibody comprises or consists of a sequence having at least 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% of identity with sequence SEQ ID NO: 74, and/or the light chain variable region of the antagonist anti-FGFR4 antibody comprises or consists of a sequence having at least

25 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% of identity with sequence SEQ ID NO: 83.

More preferably, the antagonist anti-FGFR4 antibody comprises at least one heavy chain variable region of sequence SEQ ID NO: 74 and at least one light chain variable region of sequence SEQ ID NO: 83.

Preferably, the antagonist anti-FGFR4 antibody comprises at least one heavy chain

30 that comprises or consists of a sequence encoded by a nucleotide sequence having at least 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% of identity with sequence SEQ ID NO: 76 or SEQ ID NO: 86.

Preferably, the antagonist anti-FGFR4 antibody comprises at least one light chain that comprises or consists of a sequence encoded by a nucleotide sequence having at least 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% of identity with sequence SEQ ID NO: 84 or SEQ ID NO: 88.

- 5 More preferably, the antagonist anti-FGFR4 antibody comprises at least one heavy chain comprising or consisting of sequence SEQ ID NO: 77 and at least one light chain comprising or consisting of sequence SEQ ID NO: 85.

- 10 More preferably, the antagonist anti-FGFR4 antibody comprises or consists of at least one heavy chain comprising or consisting of a sequence encoded by SEQ ID NO: 76 and at least one light chain comprising or consisting of a sequence encoded by SEQ ID NO: 84.

More preferably, the antagonist anti-FGFR4 antibody comprises or consists of at least one heavy chain comprising or consisting of sequence SEQ ID NO: 87 and at least one light chain comprising or consisting of sequence SEQ ID NO: 89.

- 15 More preferably, the antagonist anti-FGFR4 antibody comprises or consists of at least one heavy chain comprising or consisting of at least one heavy chain comprising or consisting of a sequence encoded by SEQ ID NO: 86 and at least one light chain comprising or consisting of a sequence encoded by SEQ ID NO: 88.

In the frame of the invention, an amino acid substitution may be a conservative or a non-conservative amino acid substitution.

- 20 Examples of conservative substitutions are shown in the Table 1 below.

Conservative substitutions	Type of Amino Acid
Ala, Val, Leu, Ile, Met, Phe, Trp, Tyr	Amino acids with aliphatic hydrophobic side chains
Ser, Tyr, Asn, Gln, Cys	Amino acids with uncharged but polar side chains
Asp, Glu	Amino acids with acidic side chains
Lys, Arg, His	Amino acids with basic side chains
Gly	Neutral side chain

Table 1

The expression "a sequence X differing from sequence Y by Z amino acid substitution(s)" means that said sequence X differs from sequence Y by only Z amino acid substitution(s).

For example, the expression "a sequence X differing from sequence Y by one or two amino acid substitutions" means that said sequence X differs from sequence Y by at most two amino acid substitutions, i.e. differs by only one or two amino acid substitutions.

For example, the expression "a sequence X differing from sequence Y by the amino acid substitution Z and optionally one or two additional amino acid substitution(s)" means that said sequence X differs from sequence Y by:

- only the amino acid substitution Z, or
- the amino acid substitution Z and one or two amino acid substitutions that are (is) different from amino acid substitution Z.

The expression "SEQ ID NO: X with one amino acid substitution at position Y" means a sequence differing from SEQ ID NO: X by one amino acid substitution at position Y of SEQ ID NO: X.

The field of the present invention is not limited to the antibodies comprising these sequences. In fact, all the antibodies that specifically bind to FGFR4, having an antagonistic action on this receptor, are part of the field of the present invention.

The antagonist anti-FGFR4 can either be a naked antibody or an immunoconjugate antibody.

In a particular embodiment of the invention, the antagonist anti-FGFR4 antibody is a conjugated antibody.

The antagonist anti-FGFR4 antibody may be conjugated to a cytotoxic agent.

The term "cytotoxic agent" denotes herein a substance which reduces or blocks the function or the growth of the cells, or causes destruction of the cells. A subject of the present invention is also an antagonist anti-FGFR4 antibody conjugated to a cytotoxic agent.

In one embodiment, the antibody or a binding fragment thereof can be conjugated to a drug, such as a maytansinoid, so as to form a "prodrug" which has cytotoxicity with respect to the cells expressing the antigen.

The cytotoxic agent of the present invention may be any compound which results in the death of a cell, or induces the death of a cell, or decreases cell viability in various ways. The preferred cytotoxic agents include, for example, maytansinoids and maytansinoid analogues,

taxoids, CC-1065 and CC-1065 analogues, dolastatin and dolastatin analogues, defined above. These cytotoxic agents are conjugated to antibodies, antibody fragments, functional equivalents, improved antibodies and analogues thereof, as described in the present application.

5 The conjugated antibodies may be prepared by in vitro methods. A linker group is used to link a drug or a prodrug to the antibody. Suitable linker groups are well known to those skilled in the art and include, in particular, disulphide groups, thioether groups, labile acid groups, photolabile groups, labile peptidase groups and labile esterase groups. Preferred linker groups are disulphide groups and thioether groups. For example, a conjugate can be
10 constructed by using a disulphide exchange reaction or by forming a thioether bridge between the antibody and the drug or prodrug.

 Compounds such as: methotrexate, daunorubicin, vincristine, vinblastine, melphalan, mitomycin C, chlorambucil, calicheamicin, tubulysin and tubulysin analogues, duocarmycin and duocarmycin analogues, dolastatin and dolastatin analogues, are also suitable for the
15 preparation of conjugates of the present invention. The molecules may also be linked to the antibody molecules via an intermediate molecule such as serum albumin. Doxorubicin and doxorubicin compounds, as described, for example, in patent application US 09/740991, may also be useful cytotoxic agents.

 The antibodies which are the subject of the present invention may be combined with a
20 cytotoxic molecule or compound. They may also be combined with an anti-angiogenic compound that acts on other angiogenic pathways.

Detailed description of the invention**Methods and uses for identifying patients suffering from a liver cancer who will most likely benefit from a treatment with an antagonist anti-FGFR4 antibody**

In a first aspect, the invention relates to the conjoint use of:

- the status of the single-nucleotide polymorphism (SNP) in codon 388 of FGFR4; and
- the status of activation of FGFR4 (i.e. activated vs non-activated);

as biomarkers for identifying patients suffering from a liver cancer who will most likely benefit from a treatment with an antagonist of FGFR4 selected from the group consisting of:

- an antagonist anti-FGFR4 antibody;

- a fragment thereof provided that said fragment keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4; and

- a mix of antagonist anti-FGFR4 antibodies and/or fragments of antagonist anti-FGFR4 antibodies which keep their FGFR4 receptor-binding specificity and their antagonist effect on FGFR4.

In an embodiment, the status of the SNP in codon 388 of FGFR4 is determined on both alleles of the homologous chromosomes.

In particular, the present invention relates to the above mentioned conjoint use, wherein the status of homozygosity for single-nucleotide polymorphism corresponding to a glycine in codon 388 of FGFR4 (i.e. FGFR4-388Gly allele) together with the status of activated FGFR4 indicate that the patient will most likely benefit from a treatment with said antagonist of FGFR4.

By "use as biomarkers" is meant an *in vitro* use, wherein SNP in codon 388 of FGFR4 may be determined e.g. using genotyping, sequencing, specific antibodies, probes and/or primers, and wherein activation of FGFR4 may be determined by measuring the level of expression of the protein or the phosphorylation status, for instance by using immunological detection methods such as an ELISA assay. Alternatively, as disclosed in the present specification, FGFR4 activation may be determined (i) by measuring the level of expression of ligands FGF19, FGF17 and/or FGF18, preferably at least ligand FGF19, for instance by using immunological detection methods such as an ELISA assay, and/or (ii) by detecting gene duplication of the gene coding FGF19, e.g. using sequencing, probes and/or primers.

For determining genetic variation at protein level, these methods include, without being limited to, immunochemistry, Elisa and Western blotting assays carried out with antibodies specifically detecting the mutated protein or the wild-type protein.

The level of expression of the protein may be, for instance, determined using immunological detection methods such as an ELISA assay, immunochemistry and Western blotting.

Methods for detecting genetic variation at polynucleotide level of are well-known in the art and include genotyping, PCR (e.g. RT-PCR and QRT-PCR) analysis, sequencing, ligase chain reaction (LCR), transcription-mediated amplification (TMA), strand displacement amplification (SDA), nucleic acid sequence based amplification (NASBA), Amplification Refractory Mutation System (ARMS), PCR-RFLP (restriction length fragment polymorphism) and High Resolution Melting analysis (HRM PCR).

In a second aspect, the invention relates to a method of identifying a patient suffering from a liver cancer who will most likely benefit from a treatment with an antagonist of FGFR4 selected from the group consisting of:

- an antagonist anti-FGFR4 antibody;

- a fragment of an antagonist anti-FGFR4 antibody provided that said fragment keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4; and

- a mix of antagonist anti-FGFR4 antibodies and/or fragments of antagonist anti-FGFR4 antibodies which keep their FGFR4 receptor-binding specificity and their antagonist effect on FGFR4, said method comprises:

- i) in a sample obtained from the patient, determining the single-nucleotide polymorphism (SNP) in codon 388 of FGFR4 on both alleles of the homologous chromosomes;

- ii) in a sample obtained from the patient, determining if FGFR4 is activated;

wherein homozygosity for single nucleotide polymorphism corresponding to a glycine in codon 388 of FGFR4 (i.e. FGFR4-388Gly allele) together with detection of FGFR4 activation in the sample(s) obtained from the patient indicate that the patient will most likely benefit from a treatment with an antagonist anti-FGFR4 antibody, a fragment thereof provided that said fragment keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4, or a mix of antagonist anti-FGFR4 antibodies and/or fragments of antagonist anti-FGFR4 antibodies which keep their FGFR4 receptor-binding specificity and their antagonist effect on FGFR4.

The sample used in step (i) and that of step (ii) can be the same sample, or in contrast two different samples, depending on the methods used to carry each step and the type of biological material which must be used.

Since SNP in codon 388 of the gene coding for FGFR4 is a germinal form of the gene, SNP determination / genotyping can be carried out readily on blood sample, or alternatively on a biopsy of liver tumor, or on any biological samples containing DNA or RNA.

For determining the level of activation of FGFR4, the nature of the biological sample depends on the method implemented. When the level of activation is measured by determining the phosphorylation of FGFR4 and/or the phosphorylation of proteins (e.g. kinases) involved in FGFR4 signaling pathway, the biological sample is a biopsy of liver tumor obtained from the patient to be tested. When the level of activation of FGFR4 is determined by measuring the expression level of FGF19, FGF17 and/or FGF18, the biological sample can be a blood sample, a serum sample, a plasma sample or a biopsy of liver tumor. When the level of activation of FGFR4 is determined by assessing gene duplication/amplification of FGFR4, the biological sample is preferably a biopsy of the tumor.

Methods of treating patients suffering from a liver cancer

In a third aspect, the invention provides an antagonist of FGFR4 selected from the group consisting of:

- an antagonist anti-FGFR4 antibody;
- a fragment of an antagonist anti-FGFR4 antibody provided that said fragment keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4; and
- a mix of antagonist anti-FGFR4 antibodies and/or fragments of antagonist anti-FGFR4 antibodies which keep their FGFR4 receptor-binding specificity and their antagonist effect on FGFR4;

for use for treating liver cancer in a patient in need thereof, wherein the patient is homozygous for single nucleotide polymorphism corresponding to the FGFR4-388Gly allele, and wherein FGFR4 is activated (for instance in a tumor cells or in the tumor micro-environment such as endothelial cells).

In a preferred embodiment of this aspect of the invention, the patient to be treated has been selected by a method of the invention of identifying a patient suffering from a cancer who will most likely benefit from a treatment with an antagonist anti-FGFR4 antibody.

In a fourth aspect, the invention relates to a method of treating a patient suffering from a liver cancer, said method comprising:

i) in a sample obtained from the patient, determining the single-nucleotide polymorphism (SNP) in codon 388 of FGFR4 on both alleles of the homologous chromosomes;

ii) in a sample obtained from the patient, determining if FGFR4 is activated;

5 iii) if (a) the patient is homozygous for single nucleotide polymorphism corresponding to the FGFR4-388Gly allele, and (b) FGFR4 is activated, then administering to said patient a therapeutically effective amount of an antagonist of FGFR4 selected from the group consisting of:

- an antagonist anti-FGFR4 antibody;

10 - a fragment of an antagonist anti-FGFR4 antibody provided that said fragment keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4;

- a mix of antagonist anti-FGFR4 antibodies and/or fragments of antagonist anti-FGFR4 antibodies provided that said fragments keep their FGFR4 receptor-binding specificity and their antagonist effect on FGFR4.

15

In a fifth aspect, the inventions relates to a pharmaceutical composition for the treatment of liver cancer in a patient who is homozygous for single nucleotide polymorphism corresponding to the FGFR4-388Gly allele, and whose FGFR4 is activated, the pharmaceutical composition comprises an antagonist anti-FGFR4 antibody, a fragment of an antagonist anti-FGFR4 antibody which keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4, or a mix of antagonist anti-FGFR4 antibodies and/or fragments of antagonist anti-FGFR4 antibodies provided that said fragments keep their FGFR4 receptor-binding specificity and their antagonist effect on FGFR4.

20

25 In any embodiment of the methods and uses according to the first to fifth aspects of the invention, activation of FGFR4 receptor may be readily assessed by one skilled in the art according to methods well known in the art.

Since FGFR4 receptor is activated through phosphorylation on tyrosine residues located towards the cytoplasmic domain, e.g. on Tyr⁶⁴² and Tyr⁶⁴³ which seem essential for signalling, or Tyr⁷⁵⁴, functional activation of the FGFR4 receptor may for example be assessed by measuring its phosphorylation.

30

For instance, analysis of phosphorylation of the activated FGFR4 receptor can be performed as described by Desnoyers et al., Oncogene 2007; Taylor et al., J. Clin. Invest. 2009; Li et al., PLoS ONE 2013.

Alternatively, receptor phosphorylation can be readily detected by immunocytochemistry, immunohistochemistry, ELISA assay, Western blotting, phospho-array and/or flow cytometry using antibodies which specifically recognize this modification. An ELISA assay may be used to determine the phosphorylation of FGFR4 as shown in the
5 "EXAMPLES" section of the present application. For instance phosphorylation of FGFR4 on the Tyr⁶⁴² and Tyr⁶⁴³ residues can be detected by immunocytochemistry, immunohistochemistry, ELISA, phospho-array and/or flow cytometry using monoclonal or polyclonal antibodies directed against phosphorylated Tyr⁶⁴² and Tyr⁶⁴³-FGFR4.

Functional activation of the FGFR4 receptor may also be tested by detecting the
10 presence of a phosphorylated form of a protein or a panel of proteins involved in the phosphorylation cascade triggered by FGFR4 activation. FGFR4, when associated with its ligands, e.g. FGF17, FGF18 or FGF19, in particular FGF19, mediates signaling by activating the c-Jun NH2 kinase (JNK) and Erk1/2, AKT and MAP kinase pathways (which involve P44/42 and JNK), and the STAT (signal transducer and activator of transcription) pathway.
15 Therefore activation of FGFR4 receptor can also be assessed by determining the activation of these specific pathways by detecting the phosphorylation of proteins involved in these pathways, for instance FRS2 (the immediate substrate of FGFR4), SRC (phosphorylation on Tyr⁴¹⁶ and/or Tyr⁵²⁷ when activated), P44/42, JNK, STAT3 (phosphorylation on Tyr⁷⁰⁵ and/or Ser⁷²⁷ when activated)...

Phosphorylation of proteins involved in FGFR4 signaling pathway may be assessed by
20 immunocytochemistry, immunohistochemistry, ELISA assay, Western blotting, phospho-array and/or flow cytometry using antibodies directed against the phosphorylated form of the proteins. Example of Western blotting for detecting the phosphorylated form of AKT, ERK1/2 and FRS2 are provided in the "EXAMPLES" section of the present application. Another way
25 to determine the level of FGFR4 activation is based on the determination of the level of expression of a FGFR4-specific ligand, e.g. FGF17, FGF18 or FGF19, in particular FGF19, and/or on the detection of FGF17, FGF18 and/or FGF19 gene duplication/amplification in a sample of the patient to be tested. Thus, for instance, an ELISA assay allows determining an increase in FGF19 expression in tumor biopsies or in blood samples. ELISA assay kits for
30 quantifying FGF19 are commercially available and are for instant marketed by R&D Systems (product reference DF1900), Abnova (product reference KA2207), AssayBiotechnology (product reference OK0220), GenWay Biotech Inc; (product reference GWB-SKR121), Aviscera Bioscience (product reference SK-00148-01) Gene duplication may be determined at gene sequence level by methods well known to the one skilled in the art. These methods
35 include sequencing, hybridization methods with DNA probes specific of FGF19 gene,

genotyping, and amplification methods such as quantitative polymerase chain reaction (qPCR) or a polymerase chain reaction (PCR).

In any embodiment of the methods and uses according to the first to fifth aspects of the invention, the SNP in codon 388 of FGFR4 may be determined at polynucleotide sequence level (*i.e.* gene sequence or mRNA level) and/or at protein level. The analysis may be a qualitative analysis or a quantitative measure.

Numerous methods allowing determining the presence of a genetic variation in a biological sample are well known from the one skilled in the art.

For determining genetic variation at protein level, these methods include, without being limited to, immunochemistry, Elisa and Western blotting assays carried out with antibodies specifically detecting the variant protein.

The presence of a given protein may be, for instance, determined using immunological detection methods such as an ELISA assay. The methods involve an antibody which binds specifically to the variant protein of interest, for example a monoclonal or polyclonal antibody, an antibody variant or fragments such as a single chain antibody, a diabody, a minibody, a single chain Fv fragment (sc(Fv)), a Sc(Fv)₂ antibody, a Fab fragment or a F(ab')₂ fragment, or a single domain antibody. Such antibodies are well known in the art and are commercially available. They may also notably be obtained by immunization of animals (for example rabbits, rats or mice) with the protein of interest. Antibodies may be used to determine protein expression in a range of immunological assays including competitive and non-competitive assay systems using techniques such as western blotting, immunohistochemistry/ immunofluorescence (*i.e.* protein detection on fixed cells or tissues), radioimmunoassay such as RIA (radio-linked immunoassay), ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, *e.g.* FIA (fluorescence-linked immunoassay), chemiluminescence immunoassays, ECLIA (electrochemiluminescence immunoassay) and protein A immunoassays. Such assays are routine and well known to the person skilled in the art (Ausubel et al (1994) Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York).

For determining genetic variation at gene sequence level, these methods include, without being limited to, sequencing (*e.g.* automated sequencing, microsequencing and pyrosequencing), hybridization methods with DNA probes specific of said genetic variation (*e.g.* comparative genomic hybridization (CGH), matrix-CGH, array-CGH, oligonucleotide

arrays and representational oligonucleotide microarray (ROMA)), high-throughput technologies for genotyping, and amplification methods such as quantitative polymerase chain reaction (qPCR) or a polymerase chain reaction (PCR) followed by sequencing, microsequencing, pyrosequencing or RFLP, ligase chain reaction (LCR), Amplification Refractory Mutation System (ARMS), and High Resolution Melting analysis (HRM PCR).

For determining genetic variation at mRNA level, these methods include, without being limited to, Northern blotting, a polymerase chain reaction (PCR) (e.g. reverse-transcription PCR (RT-PCR) and quantitative reverse-transcription PCR (QRT-PCR)), amplification methods such as QRT-PCR or QRT-PC followed by sequencing, microsequencing, pyrosequencing, transcription-mediated amplification (TMA), ligase chain reaction (LCR), strand displacement amplification (SDA), nucleic acid sequence based amplification (NASBA), methods such as QRT-PCR or QRT-PC for creating a DNA copy (cDNA) followed by Amplification Refractory Mutation System (ARMS) or High Resolution Melting analysis (HRM PCR).

The amplification refractory mutation system (ARMS) is an amplification strategy in which a polymerase chain reaction (PCR) primer is designed in such a way that it is able to discriminate among templates that differ by a single nucleotide residue. ARMS has also been termed allele-specific PCR or PCR amplification of specific alleles (PASA). Thus, an ARMS primer can be designed to amplify a specific member of a multi-allelic system while remaining refractory to amplification of another allele that may differ by as little as a single base from the former. The main advantage of ARMS is that the amplification step and the diagnostic steps are combined, in that the presence of an amplified product indicates the presence of a particular allele and vice versa. For routine diagnosis, this characteristic of ARMS means that it is a very time-efficient method.

High Resolution Melt (HRM) analysis is a powerful technique in molecular biology for the detection of mutations, polymorphisms and epigenetic differences in double-stranded DNA samples. HRM analysis is performed on double stranded DNA samples. Typically polymerase chain reaction (PCR) will be used prior to HRM analysis to amplify the DNA region in which the mutation of interest lies. Essentially the PCR process turns a tiny amount of the region of DNA of interest into a large amount, so the quantities are large enough for better analysis. In the tube there are now many of copies of the region of DNA of interest. This region that is amplified is known as the amplicon. After the PCR process the HRM analysis begins. The process is simply a precise warming of the amplicon DNA from around 50°C up to around 95°C. At some point during this process, the melting temperature of the amplicon is reached and the two strands of DNA separate or "melt" apart. The secret of HRM is to monitor this process happening in real-time. This is achieved by using a fluorescent dye.

The dyes that are used for HRM are known as intercalating dyes and have a unique property. They bind specifically to double-stranded DNA and when they are bound they fluoresce brightly. In the absence of double stranded DNA they have nothing to bind to and they only fluoresce at a low level. At the beginning of the HRM analysis there is a high level of fluorescence in the sample because of the billions of copies of the amplicon. But as the sample is heated up and the two strands of the DNA melt apart, presence of double stranded DNA decreases and thus fluorescence is reduced. The HRM machine has a camera that watches this process by measuring the fluorescence. The machine then simply plots this data as a graph known as a melt curve, showing the level of fluorescence vs the temperature. The melting temperature of the amplicon at which the two DNA strands come apart is entirely predictable. It is dependent on the sequence of the DNA bases. If you two samples from two different people are compared, they should give exactly the same shaped melt curve. However if one of the people has a mutation in the amplified DNA region, then this will alter the temperature at which the DNA strands melt apart. So now the two melt curves appear different. The difference may only be tiny, perhaps a fraction of a degree, but because the HRM machine has the ability to monitor this process in "high resolution", it is possible to accurately document these changes and therefore identify if a mutation is present or not.

In an embodiment, the SNP in codon 388 of FGFR4 gene is determined by sequencing the gene sequence encoding FGFR4 (or at least the gene sequence fragment where the genetic variation is supposed to be), or by sequencing the cDNA (or at least the cDNA sequence fragment where the genetic variation is supposed to be) obtained after carrying out an RT-PCR on an mRNA of FGFR4.

In another embodiment, the SNP in codon 388 of FGFR4 gene is determined by sequencing or genotyping, using pairs of primers specific for FGFR4 exon 9. In particular, for carrying out this embodiment, the forward primer may have the sequence 5'-GGCCAGTCTCACCCTGACC-3' (SEQ ID NO: 69), the reverse primer may have the sequence 5'-TGCTGGAGTCAGGCTGTAC-3' (SEQ ID NO: 70).

Kits according to the invention

The invention provides kits that are useful in the above methods and uses of the invention.

A sixth aspect of the invention relates to a kit for determining the SNP in codon 388 of FGFR4 gene.

Such a kit comprises means for determining the SNP in codon 388 of FGFR4 gene.

In particular, such kit comprises means for determining the homozygosity for SNP corresponding to a glycine in codon 388 of FGFR4 (i.e. FGFR4-388Gly allele).

A seventh aspect of the invention relates to a kit for identifying a patient suffering from a cancer who will most likely benefit from a treatment with an antagonist FGFR4 antibody or a fragment of an antagonist anti-FGFR4 antibody provided that said fragment keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4.

Such a kit comprises:

(i) means for determining the SNP in codon 388 of FGFR4 gene;

(ii) means for determining the activation, or level of activation, of FGFR4; and

(iii) optionally means for measuring the expression level of FGF19, FGF17 and/or FGF18.

In particular, such kit comprises means for determining the homozygosity for SNP corresponding to a glycine in codon 388 of FGFR4 (i.e. FGFR4-388Gly allele).

For determining the SNP in codon 388 of FGFR4 at protein level, such means include, without being limited to, antibodies specific for FGFR4-388Gly (i.e. FGFR4 wherein the amino acid in position 388 is a glycine) and/or antibodies specific for FGFR4-388Arg (i.e. FGFR4 wherein the amino acid in position 388 is an arginine).

For determining SNP in codon 388 of FGFR4 at gene sequence level, such means include, without being limited to, pair of primers suitable for sequencing or PCR amplification of FGFR4 gene, or of a fragment of FGFR4 gene provided that said fragment comprises the SNP in codon 388, and DNA probes specific of the SNP.

Means for determining the activation of FGFR4 includes, for instance, antibodies which specifically recognize the phosphorylated form of FGFR4, in particular antibodies specifically directed against FGFR4 wherein the tyrosine at position 642 or at position 643 is phosphorylated. Means for determining the activation of FGFR4 also includes, for instance, antibodies which specifically recognize the phosphorylated form of proteins involved in the phosphorylation cascade triggered by FGFR4 activation. Such means include when antibodies specific for the phosphorylated form of FRS2 (the immediate substrate of FGFR4), c-Jun NH2 kinase (JNK), Erk1/2, AKT, P44/42, STAT3 (phosphorylated on Tyr705 and/or Ser727 when activated), SRC (phosphorylated on Tyr416 and/or Tyr527 when activated).

Means for measuring the expression level of FGF19, FGF17 and FGF18 can be means for measuring the expression level of FGF19, FGF17 and/or FGF18 protein(s), and/or the expression of FGF19, FGF17 and/or FGF18 mRNA(s). For instance, for measuring expression of FGF19 at protein level, such means include antibodies specific for FGF19.

Means for measuring expression at mRNA level include pair of primers specific for FGF19 and reagents allowing the detection of HGF mRNA by real-time quantitative-PCR. The means for measuring the expression level of FGF19 may also include reagents such as e.g. reaction, hybridization and/or washing buffers. The means may be present, e.g., in vials or microtiter plates, or be attached to a solid support such as a microarray as can be the case for primers and probes.

Such means can be labeled with detectable compound such as fluorophores, enzymes like peroxydases or phosphatases, or radioactive compounds.

For example, the probe or the antibody specifically binding to FGFR4 gene or FGFR4 protein, respectively, may be labeled with a detectable compound.

When the kit comprises an antibody, the kit may further comprise a secondary antibody, labeled with a detectable compound, which binds to an unlabelled antibody specifically binding to the target, for instance to FGFR4, a phosphorylated form of FGFR4 or a specific mutant of FGFR4.

The means may be present, e.g., in vials or microtiter plates, or be attached to a solid support such as a microarray as can be the case for primers and probes.

Further, the kit can comprise instructions for the use of said kit in identifying a patient suffering from a cancer who will most likely benefit from a treatment with an antagonist FGFR4 antibody.

The kit can also comprise a "reference level of activation of FGFR4". This reference level may correspond to the basal level of activation in a given biological sample. The reference level of activation of FGFR4 may also be the level of activation of a given cell or cell line cultured without FGFR4 ligands. The reference level of activation of FGFR4 may be also a value obtained by determining the activation level of FGFR4 measured, for instance, in biological samples from a population of healthy subjects (i.e. subjects who do not suffer from a cancer).

A eighth aspect of the invention relates to an article of manufacture comprising, or consisting of :

- a packaging material;

- at least one antagonist anti-FGFR4 antibody or a fragment of an antagonist anti-FGFR4 antibody provided that said fragment keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4;

- a label or package insert contained with said packaging material indicating that said
5 at least one antagonist anti-FGFR4 antibody or fragment of an antagonist anti-FGFR4 antibody provided that said fragment keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4 will most likely be effective in treatment of liver cancer in patient suffering from cancer who is homozygous for single nucleotide polymorphism corresponding to the FGFR4-388Gly allele, and whose FGFR4 is activated.

10

The antagonist anti-FGFR4 antibody, as well as the fragment of an antagonist anti-FGFR4 antibody provided that said fragment keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4, of the first to eighth aspects of the invention, used in the above recited method or use for treating a patient suffering from a cancer are preferably
15 provided in a pharmaceutical composition, i.e. a pharmaceutically acceptable carrier, excipient or diluent which is not prejudicial to the patient to be treated.

Pharmaceutically acceptable carriers and excipient that may be used in the compositions of this invention include, but are not limited to, ion exchangers, alumina, aluminium stearate, lecithin, self-emulsifying drug delivery systems (SEDDS) such as d-a-
20 tocopherol polyethyleneglycol 1000 succinate, surfactants used in pharmaceutical dosage forms such as Tweens or other similar polymeric delivery matrices, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium
25 hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

As appreciated by skilled artisans, compositions are suitably formulated to be
30 compatible with the intended route of administration. Examples of suitable routes of administration include parenteral route, including for instance intramuscular, subcutaneous, intravenous, intraperitoneal or local intratumoral injections. The oral route can also be used, provided that the composition is in a form suitable for oral administration, able to protect the active principle from the gastric and intestinal enzymes.

Preferably, the pharmaceutical compositions contain carriers that are pharmaceutically acceptable for an injectable formulation. They may in particular be sterile, isotonic, saline solutions (monosodium phosphate, disodium phosphate, sodium chloride, potassium chloride, calcium chloride or magnesium chloride etc., or mixtures of such salts), or dry, in particular lyophilized, compositions which by means of the addition, as appropriate, of sterilized water or physiological saline, can form injectable solutes.

Further, as previously indicated the amount of antagonist anti-FGFR4 antibodies and fragments of antagonist anti-FGFR4 antibodies which keep its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4, used in the above recited method or use for treating a patient suffering from a cancer, is a therapeutically effective amount.

The exact amount of compounds antagonist anti-FGFR4 antibodies and fragments of antagonist anti-FGFR4 antibodies which keep its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4 to be used and the composition to be administered will vary according to the age and the weight of the patient being treated, the stage of the cancer to be treated, the mode of administration, the frequency of administration as well as the other ingredients in the composition which comprises the antagonist anti-FGFR4 antibodies drugs. Such concentrations can be routinely determined by those of skilled in the art.

The amount of antagonist anti-FGFR4 antibodies and fragments of antagonist anti-FGFR4 antibodies which keep its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4 actually administered will typically be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual anti-cancer agents administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, as well as the possibility of co-usage with other agents, etc.

The total dose required for each treatment may be administered by multiple doses or in a single dose.

Purely by way of illustration, the administered amount of antagonist anti-FGFR4 antibodies and fragments of antagonist anti-FGFR4 antibodies which keep its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4 may be, e.g., from about 0.1 µg/kg to up to about 100 mg/kg or more, preferably from about 0.5 mg/kg to up to about 20 mg/kg, depending on the factors mentioned above. In other embodiments, the dosage may range from 1 µg/kg up to about 100 mg/kg; or 5 µg/kg up to about 100 mg/kg; or 10 µg/kg up to about 100 mg/kg. Due to the hyperproliferative nature of cancer, a single dose of antibody or fragment thereof may not accomplish a complete anti-cancer effect. Therefore, prolonged treatment involving multiple doses of a therapeutic agent may be required.

Depending on the intended route of delivery, the compounds may be formulated as liquid (e.g., solutions, suspensions), solid (e.g., pills, tablets, suppositories) or semisolid (e.g., creams, gels) forms.

5 In a first implementation of the first to eighth aspects of the invention according to any one of the embodiments, the liver cancer is a hepatocellular carcinoma.

In a second implementation of the first to fifth aspects of the invention according to any one of the embodiments and/or according to the first implementation, the antagonist anti-
10 FGFR4 antibody of the invention comprises:

- i) six CDRs having the amino acid sequences SEQ ID Nos: 5, 6, 7, 8, 9, 10; or
- ii) six CDRs having the amino acid sequences SEQ ID Nos: 11, 12, 13, 14, 15, 16; or
- iii) six CDRs having the amino acid sequences SEQ ID Nos: 17, 18, 19, 20, 21, 22; or
- iv) six CDRs having the amino acid sequences SEQ ID Nos: 23, 24, 25, 26, 27, 28; or
- 15 v) six CDRs having the amino acid sequences SEQ ID Nos: 29, 30, 31, 32, 33 or 34; or
- vi) six CDRs the sequences of which differ from their corresponding respective sequences mentioned at point (i), (ii), (iii), (iv) or(v) respectively, at most by one or two amino acids, provided that the antibody keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4.

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In a third implementation of the first to fifth aspects of the invention according to any one of the embodiments and/or according to the first implementation, the antagonist anti-FGFR4 antibody comprises a heavy chain variable region and a light chain variable region encoded by nucleotide sequences chosen from the group consisting of:

- 25 (i) SEQ ID Nos. 39 and 41;
- (ii) SEQ ID Nos. 53 and 55;
- (iii) SEQ ID Nos. 57 and 59;
- (iv) SEQ ID Nos. 61 and 63, and
- (v) SEQ ID Nos. 65 and 67;
- 30 (vi) a heavy chain variable region and a light chain variable region encoded by nucleotide sequences at least 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to (i), (ii),

(iii), (iv) or (v), provided that the antibody keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4.

In a fourth implementation of the first to fifth aspects of the invention according to any one of the embodiments and/or according to the first implementation, the antagonist anti-FGFR4 antibody comprises polypeptide sequences chosen from the group consisting of:

(i) SEQ ID No. 36 and SEQ ID No. 38; or

(ii) SEQ ID Nos. 40 and 42; or

(iii) SEQ ID No. 54 and SEQ ID No. 56; or

(iv) SEQ ID No. 58 and SEQ ID No. 60; or

(v) SEQ ID No. 62 and SEQ ID No. 64; or

(vi) SEQ ID No. 66 and SEQ ID No. 68; or

(vii) two polypeptide sequences at least 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to polypeptides of (i), (ii), (iii), (iv), (v) or (vi), provided that the antibody keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4.

In a fifth implementation of the first to fifth aspects of the invention according to any one of the embodiments and/or according to the first implementation, the antagonist anti-FGFR4 antibody is chosen from the group consisting of antibody 40-12, antibody 8, antibody 31, antibody 33, antibody 36 and antibody LD1, or a humanized antagonist anti-FGFR4 antibody thereof.

In a sixth implementation of the first to fifth aspects of the invention according to any one of the embodiments and/or according to the first implementation, the antagonist anti-FGFR4 antibody comprises:

- a CDRH1 comprising or consisting of sequence SEQ ID NO: 71, SEQ ID NO: 5, SEQ ID NO: 95 or a sequence differing from one of these sequences by one or two amino acid substitutions,
- a CDRH2 comprising or consisting of sequence SEQ ID NO: 72, SEQ ID NO: 6, SEQ ID NO: 96 or a sequence differing from one or two amino acid substitutions, provided that none of said one or two amino acid substitution(s) is located at position 11 of SEQ ID NO: 72,

- a CDRH3 comprising or consisting of sequence SEQ ID NO: 73 or SEQ ID NO: 7, or a sequence differing from one of these sequences by one or two amino acid substitutions,
- 5 - a CDRL1 comprising or consisting of sequence SEQ ID NO: 80, SEQ ID NO: 94 or a sequence differing from one of these sequences by one or two amino acid substitutions provided that none of said one or two amino acid substitution(s) is located at position 8 of SEQ ID NO: 80 or at position 5 of SEQ ID NO: 94,
- a CDRL2 comprising or consisting of sequence SEQ ID NO: 81 or a sequence differing from one of this sequence by one or two amino acid substitutions,
10 provided that none of said one or two amino acid substitution(s) is located at position 4 of SEQ ID NO: 81, and
- a CDRL3 comprising or consisting of sequence SEQ ID NO: 82 or a sequence differing from one of this sequence by one or two amino acid substitutions provided that none of said one or two amino acid substitution(s) is located at
15 position 4 of SEQ ID NO: 82.

In a seventh implementation of the first to fifth aspects of the invention according to any one of the embodiments and/or according to the first, the antagonist anti-FGFR4 antibody comprises:

- 20 - a CDRH1 comprising or consisting of sequence SEQ ID NO: 71, SEQ ID NO: 5 or SEQ ID NO: 95,
- a CDRH2 comprising or consisting of sequence SEQ ID NO: 72,
- a CDRH3 comprising or consisting of sequence SEQ ID NO: 73 or SEQ ID NO: 7,
- 25 - a CDRL1 comprising or consisting of sequence SEQ ID NO: 80 or SEQ ID NO: 94,
- a CDRL2 comprising or consisting of sequence SEQ ID NO: 81, and
- a CDRL3 comprising or consisting of sequence SEQ ID NO: 82.

30 In an eighth implementation of the first to fifth aspects of the invention according to any one of the embodiments and/or according to the first, the antagonist anti-FGFR4 antibody comprises at least one heavy chain variable region of sequence SEQ ID NO: 74 and at least one light chain variable region of sequence SEQ ID NO: 83.

35 In a nine implementation of the first to fifth aspects of the invention according to any one of the embodiments and/or according to the first, the antagonist anti-FGFR4 antibody

comprises at least one heavy chain comprising or consisting of sequence SEQ ID NO: 77 and at least one light chain comprising or consisting of sequence SEQ ID NO: 85.

Throughout the present application, the references to entries of public databases refer to the entries in force on August 16, 2013. Further, throughout this application, various references are cited. The disclosures of these references are hereby incorporated by reference into the present disclosure.

The present invention will be further illustrated by the additional description which follows, which refer to examples which show that antagonist anti-FGFR4 antibodies have an inhibitory activity on proliferation of tumor cells when FGFR4 is activated and that the tumor cells are homozygous for the gene coding a FGFR4 with a Glycine at position 388. It should be understood however that the invention is defined by the claims, and that these examples are given only by way of illustration of the invention and do not constitute in anyway a limitation thereof.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 illustrates the genotyping for the identification of the status of FGF-R4 SNP at the position 388 for several human cell lines of hepatocarcinoma. Hep3B, SNU182, SNU398 and THLE2 are homozygous for Gly388 allele.

Figure 2 illustrates the response of *in vitro* proliferation of several HCC cell lines in a Matrigel® matrix in presence of 40-12 or LD1 antibodies.

Figure 3 illustrates the activity of two others antibodies (Ab33 and Ab36) on HCC cell line proliferation (Hep3B and HepG2) in Matrigel® matrix

Figure 4 illustrates the studies of FGFR4 signaling (FGFR4, Erk1/2, AKT and FRS2 phosphorylation) in Hep3B cells stimulated with FGF19 and treated with a FGFR tyrosine kinase inhibitor (PD173074), 40-12 or LD1 antibodies.

Figure 5 illustrates the studies of FGFR4 signaling (FGFR4, Erk1/2, AKT and FRS2 phosphorylation) in HepG2 cells stimulated with FGF19 and treated with a FGFR tyrosine kinase inhibitor (PD173074), 40-12 or LD1 antibodies.

Figure 6 illustrates the studies of FGFR4 signaling (FGFR4, Erk1/2, AKT and FRS2 phosphorylation) in HUH7 cells stimulated with FGF19 and treated with a FGFR tyrosine kinase inhibitor (PD173074), 40-12 or LD1 antibodies.

Figure 7 illustrates the response of pFGFR4 and *in vitro* proliferation of 3 HCC cell lines (Hep3b, SNU182 and SNU398) stimulated by FCS in a Matrigel® matrix in presence of 40-12 antibody

Figure 8 illustrates the FGF19 release in the culture supernatant of HCC cell lines and the corresponding pFGFR4.

Figure 9 illustrates the dosage of FGF19 from culture supernatant of Hep3 cell or lysates from Hep3b orthotopic tumors in SCID mice vs healthy liver

Figure 10 illustrates the *in vivo* activity of 40-12 on tumor weight in an orthotopic model of hep3B HCC in SCID mice after 2 treatments by s.c. route at 25mg/kg once a week.

Figure 11 illustrates the *in vivo* activity of 40-12 on tumor weight in an orthotopic model of hepG2 HCC in SCID mice after 2 treatments by s.c. route at 25mg/kg once a week.

EXAMPLES:

MATERIALS AND METHODS

FGFR4 genotyping at the position 388: the protocol for the identification of FGFR4 SNP status at the position 388 (Gly: G or Arg: R) is originated from the PhD dissertation of Gaughlofer from the University of Vienna defended in 2010. Shortly, allele determination is realized on genomic DNA or cDNA classically isolated. A region of the exon 9 of FGFR4 is amplified by PCR between sens primer GGCCAGTCTCACCACTGACC (SEQ ID NO. 69) and forward primer TGCTGGAGTCAGGCTGTAC (SEQ ID NO. 70). The 298bp amplified PCR fragment is then restricted by MspI enzyme. Depending on the polymorphism, the digestion pattern is specific: 31-107-160bp for the Gly388 allele, 107-191bp for the Arg388 allele. Heterozygosity at this position will give 31-107-160-191bp.

3D-proliferation of human HCC cell lines in a matrigel® matrix: in white 96-wells plate with clear bottom (Interchim Fluoprobes FP BA7880) put on ice, in each well, dispense 40µL on a mix composed by 20µL of cold liquid Matrigel® and 20µL of a cell suspension in full culture medium (1.25 10⁵ cells/mL for Hep3b, 0.5 10⁵ cells/mL for HepG2, HUH7, SNU182 and SNU398). Incubate 1h at 37°C in an incubator (90% humidity, 5% CO₂). Then, add 90µL of full culture medium and 10µL of 10x concentrated compounds to be tested. Incubate 10 days at 37°C in an incubator (90% humidity, 5% CO₂). At day 6 and day 8, aspirate the medium and replace with 90µL of fresh one and 10µL of 10x-concentrated compounds. Cells

proliferation was assessed by the addition in the medium of 10µL of alamarBlue® ready to use reagent (Invitrogen, DAL1025) in each well. Following a 2h-incubation period, the fluorescence (Ex:530nm, Em:590nm) were quantified with a microplate reader Magellan Infinite M200. For each condition, the test was performed with 5 replicates.

5 Signaling experiments in human HCC cell lines: HCC cell lines were grown in T75 flask up to 80% confluency. Then, cells are starved for 24h in 9mL of full medium without serum. 0.5 mL of 20x concentrated antibodies diluted in the equilibrated starvation medium (600µg/mL) were added to the cells and then, cells were stimulated for 10 min by the addition of 0.5 mL of 20x concentrated FGF19 (in-house production) also diluted in the equilibrated starvation
10 medium (2µg/mL). Then, following a 2 washing step with cold PBS, 500µL of ice cold lysis buffer RIPA (Sigma, R0278) containing anti-proteases (Halt Protease, Thermoscientific, I861281) and 2mM orthovanadate were added for 30min on ice. Lysed cells were removed using a scrapper and cell lysats were recovered in microcentrifuge tubes. Following a 10 min centrifugation at 13000 rpm and 4°C, supernatant were collected in a new microcentrifuge
15 tubes.

For FGFR4 phosphorylation ELISA dosage, 96-wells plates (NUNC maxisorp, 442404) were overnight coated with a capture anti-FGFR4 specific antibody at 4°C. Wells were rinsed 5 times with PBS+0.05% Tween 20. Then a 1h saturation step is performed with 200µL per well of PBS+0.05% Tween 20 supplemented with 5% of normal goat serum (Invitrogen
20 50062Z). Wells were rinsed 5 times with PBS+0.05% Tween 20. Protein lysates were diluted in IC diluent 12 (R&D, DYC002, 895891) to reach a final concentration of 1500µg/mL. 100µL of this lysate is added into well and incubated for 4h at 4°C under gentle agitation. Then, wells were rinsed 5 times with PBS+0.05% Tween 20. 100µL of anti-pFGFRs detection antibody (Santa Cruz Biotechnology, Sc30262) 1/1000 diluted in IC diluent 14 (R&D DY995,
25 841380) were added in each well and overnight incubated at 4°C. Wells were rinsed 5 times with PBS+0.05% Tween 20. 100µL of HRP-conjugated secondary goat anti-rabbit antibody (Cell Signaling technology, CST7074) 1/2000 diluted in IC diluent 14 (R&D DY995, 841380) were incubated in each well for 1h at 4°C. Wells were rinsed 5 times with PBS+0.05% Tween 20. Detection of FGFR4 phosphorylation was performed by the addition of 100µL of HRP
30 substrat in each well (Uptima, UP664781) for 10min under gentle agitation and protected from light. Then, 100µL of stop solution were added (Uptima, UPS29590). Optical density is quantified at 450nm with a Tecan Infinite M200.

For Erk (p42/p44), AKT and FRS2 phosphorylations, following a denaturation step in Laemmli buffer, total protein lysates were separated on polyacrylamide gels (BioRad) and
35 subsequently transferred onto nitrocellulose membranes (iBlot, Invitrogen). Following incubation with 5% non-fat milk powder in TBS Tween20 0.05%, the membranes were

incubated overnight at 4°C with following antibodies: phospho-AKT (Cell Signaling Technology 4058), phospho-Erk (Cell Signaling Technology 4377) and phospho-FRS2 (Cell Signaling Technology 3864) were overnight incubated at 4°C at a dilution of 1:1000 in TBS, Tween20 0.05%, BSA 0.2%. Following a 3-times washing step in TBS, membranes were
5 incubated with HRP-conjugated secondary goat anti-rabbit antibody (Cell Signaling technology, CST7074) is overnight incubated at 4°C at a dilution of 1:1000 in TBS, Tween20 0.05%, BSA 0.2%.

FGF19 dosage: human FGF19 in biological samples (cell culture supernatants and tumor
10 lysates) was quantified using commercially available quantikine® immunoassay (R&D system, DF1900) following purchaser recommendations.

Protein sample preparation from Hep3b tumors: For sample preparation of tumors for ELISA dosages, the tumor was removed from the liver as fast as possible as soon as the animal has just been anesthetized and was transferred in a cryotube. The cryotube was plunged into
15 the liquid nitrogen. The sample was then pulverized in a mortar bowl that will have been cooled at least 10 minutes in the liquid nitrogen (idem for the piston which serves for crushing). The organ was crush with the hammer by giving strong knocks (at least 4 times). The “powder of organ” was get back as fast as possible (avoid that it defrosts) in an eppendorf tube. For lysis, the powder of organ was re-suspended in RIPA 1X supplemented
20 by proteases inhibitors for lysing 20 minutes in the ice before centrifugation 10 minutes at 4°C at 10000 rpm. Then protein samples were subjected to ELISA dosage.

HCC tumors induction in SCID mice: female SCID mice (Charles River Laboratory, France) are anesthetized (mixture of ketamine/xylazine) and the zone of section is shaved. The skin is cleaned and disinfected by betadine® before perform a sub-costal section about 1cm of
25 the skin and the muscular wall. The side left lobe of the liver is delicately explained. A volume of 50µl of the cell suspension mixed with Matrigel® containing $5 \cdot 10^5$ Hep3b cells is injected in the left lobe of the liver. The needle is slowly removed to avoid any leak of cells. Cotton sterile is applied during one minute to the site of injection then a scrap of haemostatic gauze is placed there, to avoid a bleeding. The lobe is replaced in the abdominal cavity. The
30 peritoneum is closed with some biological glue then the wound is closed with hooks. Mice bearing an orthotopic hep3B tumor were treated from day 21 post cell injection with the 40-12 antibody at the dose of 25mg/kg one a week by s.c. route or with s.c. injection of PBS for the control group. FGFR-4 clone 40-12 in solution in PBS at the concentration of 2.5 mg/ml was sub cutaneously injected once a week in the SCID mice at the dose of 10ml/kg. The day

of the end of experiment (D35), the animals were sacrificed for necropsy. The left lobe with the tumor was weighted.

To induce hepG2 HCC in SCID mice, a quite similar protocol than for Hep3b is used. 1×10^6 HepG2 cells were injected and the first day of the treatment with the 40-12 is D14. Experiment was stopped at D28.

Example 1: Genotyping of FGFR4 alleles at the position 388

Genomic DNA from different human HCC cell lines were analysed for the status of FGFR4 SNP at the position 388. Hep3b, SNU182, SNU398 and THLE2 are homozygous for the glycine allele (Figures 1B and 1C), HepG2 and HUH7 are homozygous for the arginine allele (Figure 1B) and SNU475 are heterozygous (Figure 1C).

This PCR-RFLP carried out here on genomic DNA samples, may also be easily perform on any DNA samples such as cDNA coming from any DNA-containing biological samples from the patient because the studied polymorphism at the position 388 is germinal and not tumor-induced.

Example 2: Evaluation of anti-FGFR4 antibodies inhibitory activity on 3D-proliferation of human HCC cell lines

Human HCC cells Hep3b (Gly388/Gly388), HepG2 (Arg388/Arg388) and HUH7 (Arg388/Arg388) were embedded in a matrigel® matrix for 10 days in a full medium. Cells proliferation in a tridimensional matrix was assessed using alamarBlue® viability assay in the presence or absence of increasing doses of 40-12 or DL1 anti-FGFR4 antibodies. Both exhibit anti-proliferative effect on Hep3b while no inhibitory activity was observed on HepG2 and HUH7 (Figure 2). These results suggest a specificity of action for the glycine allele of FGFR4.

In the same way, 2 other in-house anti-FGFR4 antibodies (Ab33 and Ab36) were evaluated in 3D-proliferation of Hep3b and HepG2. Both only inhibit Hep3b proliferation (Figure 3) confirming the specificity of action of anti-FGFR4 antibodies targeting the extracellular domain of the receptor for the Gly³⁸⁸ polymorphism.

These results clearly indicate that the response to anti-FGFR4 antibodies treatment is linked to the polymorphism of FGFR4 at the position 388, thus underlining the importance of patient genotyping before any treatment with anti-FGFR4 antibodies.

Example 3: Evaluation of anti-FGFR4 antibodies on FGFR4-dependent signaling in human HCC cell lines

In order to confirm non-inhibitory ability of anti-FGFR4 antibodies when the FGFR4 SNP at the position 388 is an arginine, signaling experiments were performed on human HCC cell lines to study FGFR4-dependent signaling in HepG2 and HUH7.

First, to confirm efficacy on Gly³⁸⁸ allele, signaling experiments were carried out on Hep3b. In this cell line, FGF19 stimulation leads to an activation of FGFR4, observed through its phosphorylation (figure 4A), and downstream dependent signaling cascades FRS2, Erk and AKT (Figures 4B-E). All these pathways are reduced upon anti-FGFR4 antibodies treatment, as it was observed with the modest FGFR4-TK inhibitor PD173074 (Figures 4A-E).

Conversely, in HepG2 cells, despite a weak activation of the phosphorylation of FGFR4 following FGF19 stimulation, antibodies not only didn't block this induction but seems to stimulate this phosphorylation (Figure 5A). This increased phosphorylation is also noticed for FRS2 (Figures 5B and 5D), the direct relay between FGFR and SH2 domain-containing proteins including Grb2 and SHP-2, mediating downstream signalling. PD173074 seems to be inefficient on these cells (Figures 5A-D) and AKT is not phosphorylated (Figures 5B-C).

For HUH7, high FGFR4 phosphorylation level is observed at the basal stage and additional activation was not shown following FGF19 stimulation (Figure 6A). Nevertheless, PD173074 moderately reduces FGFR4 phosphorylation while anti-FGFR4 antibodies did not (Figure 6A). High phosphorylation of FGFR4 in these cells leads to a significant basal activation of Erk and AKT. PD173074 treatment, on these two signaling proteins, inhibited their basal phosphorylation (Figures 6B-D) while 40-12 seems to slightly increase Erk and AKT activation, when LD1 is clearly inactive (Figures 6B-D). However, on FRS2 phosphorylation, for which the basal level is quite low, anti-FGFR4 antibodies increased its phosphorylation level (Figures 6B and 6E).

All together, these results confirmed the potent efficacy of such anti-FGFR4 antibodies on cells homozygous for the glycine form of FGFR4 and a non-inhibitory ability on cells expressing the arginine one.

These data underline that FGFR4 genotyping at the position 388 is mandatory before any treatment with anti-FGFR4 antibodies because administration of such molecules to patients with Arg388 allele would be at best inefficient, at worst deleterious.

Example 4: Evaluation of anti-FGFR4 antibody 40-12 function of FGFR4 phosphorylation level in human HCC cell lines homozygous for the Gly³⁸⁸ allele of FGFR4.

First, on the 3 HCC cell lines identify for their homozygosity on allele Gly³⁸⁸ of FGFR4 (Figures 1B-C), the basal level of FGFR4 phosphorylation was studied on serum-growing cells. In SNU398, this level is quite close to the background indicating a negligible FGFR4 activation (Figure 7A). In SNU182, the basal activation of FGFR4 is modest and it is more pronounced in Hep3b (Figure 7A). Then, 3D-proliferation assay in matrigel® matrix was performed with these 3 HCC cell lines in order to evaluate anti-FGFR4 antibody 40-12 efficacy on each ones. 40-12 inhibits Hep3b proliferation, tends to reduce SNU182 growth and is ineffective on SNU398 (Figures 7B-D). These data suggest that in cells homozygous for Gly388 anti-FGFR4 antibody potency is strongly dependent on the phosphorylation status of FGFR4 and that, following SNP determination, evaluation of FGFR4 activation is necessary to determine patient susceptibility to anti-FGFR4 antibodies treatment.

Example 5: Evaluation of relationship between FGF19 synthesis and FGFR4 activation in HCC cell lines *in vitro* and *in vivo*

The amount of FGF19 produced by HCC cell lines was assessed in culture supernatants of Hep3b, HepG2, HUH7, SNU182 and SNU398. In the same condition, FGFR4 phosphorylation level was also estimated by ELISA dosage. HUH7 and Hep3b exhibit the highest FGFR4 activation level (Figure 8B). Interestingly, these 2 cell lines also produce the largest amount of FGF19 in cell medium (Figure 8A). When orthotopically implanted in SCID mice, Hep3b form tumors in which FGF19 was produced (Figure 9A) leading to a strong phosphorylation of FGFR4 compared to non-bearing tumor liver (Figure 9B). These results support the main role of FGF19 production in HCC tumor for FGFR4 activation.

Example 6: Evaluation of 40-12 efficacy on orthotopically implanted Hep3b (Gly³⁸⁸/Gly³⁸⁸) and HepG2 (Arg³⁸⁸/Arg³⁸⁸) HCC tumors in SCID mice

Mice bearing Hep3b and HepG2 tumors were treated once a week with 40-12 at 25mg/kg for 2 weeks. This treatment induced no significant effect on the mice body weight at the end of the experiment (Figure 10B and 11B). In the Hep3b model, at D35, the mean tumor weight of the tumors in the control group was 1723 ±289 mg. Treatment with the 40-12 antibody induced a significant reduction of the tumor weight, the treated mice showing a mean tumor weight of 704 ±121mg (51% of reduction vs control group, p<0.05, Figure 10A).

Conversely, in the HepG2 mouse model, at D28 post cell injection, the mean tumor weight in control group (PBS treated) was 456 ± 89 mg. The tumors in the group treated with the 40-12 antibody show a mean tumor weight of 1240 ± 269 mg (272% of increased vs control group, $p < 0.05$, Figure 11A).

- 5 These results confirm *in vitro* data on the specific inhibitory activity of the 40-12 only on the glycine allele of FGFR4 at the position 388, and strongly support that patient which can benefit from treatment with anti-FGFR4 antibodies should be tested for their polymorphism at the position 388 of FGFR4 prior any treatments. This treatment will be efficient for patients homozygous for Gly388 allele and might be deleterious for patients bearing Arg388 ones.

CLAIMS

1. Use of:

- the status of the single-nucleotide polymorphism (SNP) in codon 388 of FGFR4; and
- the status of activation of FGFR4 ;

as conjoint biomarkers for identifying patients suffering from a liver cancer who will most likely benefit from a treatment with an antagonist of FGFR4 selected from the group consisting of:

- an antagonist anti-FGFR4 antibody;
- a fragment of an antagonist anti-FGFR4 antibody provided that said fragment keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4; and
- a mix of antagonist anti-FGFR4 antibodies and/or fragments of antagonist anti-FGFR4 antibodies which keep their FGFR4 receptor-binding specificity and their antagonist effect on FGFR4.

2. Use according to claim 1, wherein the status of homozygosity for single-nucleotide polymorphism corresponding to a glycine in codon 388 of FGFR4 (i.e. FGFR4-388Gly allele) together with the status of activated FGFR4 indicate that the patient will most likely benefit from a treatment with said antagonist of FGFR4.

3. Use according to claim 1 or 2, wherein the liver cancer is hepatocellular carcinoma.

4. A method of identifying a patient suffering from a liver cancer who will most likely benefit from a treatment with an antagonist of FGFR4 selected from the group consisting of:

- an antagonist anti-FGFR4 antibody;
- a fragment of an antagonist anti-FGFR4 antibody provided that said fragment keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4; and
- a mix of antagonist anti-FGFR4 antibodies and/or fragments of antagonist anti-FGFR4 antibodies which keep their FGFR4 receptor-binding specificity and their antagonist effect on FGFR4, said method comprises:

i) in a sample obtained from the patient, determining the single-nucleotide polymorphism (SNP) in codon 388 of FGFR4 on both alleles of the homologous chromosomes;

- ii) in a sample obtained from the patient, determining if FGFR4 is activated;

wherein homozygosity for single nucleotide polymorphism corresponding to a glycine in codon 388 of FGFR4 (i.e. FGFR4-388Gly allele) together with detection of FGFR4 activation in the sample(s) obtained from the patient indicate that the patient will most likely benefit from a treatment with said antagonist of FGFR4.

5. Method according to claim 4, wherein the liver cancer is hepatocellular carcinoma.

6. An antagonist of FGFR4 selected from the group consisting of:

- an antagonist anti-FGFR4 antibody;

a fragment of an antagonist anti-FGFR4 antibody provided that said fragment keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4; and

- a mix of antagonist anti-FGFR4 antibodies and/or fragments of antagonist anti-FGFR4 antibodies which keep their FGFR4 receptor-binding specificity and their antagonist effect on FGFR4;

for use for treating liver cancer in a patient in need thereof, wherein the patient is homozygous for single nucleotide polymorphism corresponding to the FGFR4-388Gly allele, and wherein FGFR4 is activated.

7. Use according to claim 6, wherein the liver cancer is hepatocellular carcinoma.

8. A method of treating a patient suffering from a liver cancer, said method comprising:

- i) in a sample obtained from the patient, determining the single-nucleotide polymorphism (SNP) in codon 388 of FGFR4 on both alleles of the homologous chromosomes;

- ii) in a sample obtained from the patient, determining if FGFR4 is activated;

iii) if (a) the patient is homozygous for single nucleotide polymorphism corresponding to the FGFR4-388Gly allele, and (b) FGFR4 is activated, then administering to said patient a therapeutically effective amount of an antagonist of FGFR4 selected from the group consisting of:

- an antagonist anti-FGFR4 antibody;

- a fragment of an antagonist anti-FGFR4 antibody provided that said fragment keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4;

- a mix of antagonist anti-FGFR4 antibodies and/or fragments of antagonist anti-FGFR4 antibodies which keep their FGFR4 receptor-binding specificity and their antagonist effect on FGFR4.

9. Method according to claim 8, wherein the liver cancer is hepatocellular carcinoma.

10. A kit for identifying a patient suffering from a cancer who will most likely benefit from a treatment with an antagonist FGFR4 antibody or a fragment of an antagonist anti-FGFR4 antibody provided that said fragment keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4, said kit comprising:

(i) means for determining the SNP in codon 388 of FGFR4 gene;

(ii) means for determining the activation, or level of activation, of FGFR4; and

(iii) optionally, means for measuring the expression level of FGF19, FGF17 and/or FGF18.

11. A kit according to claim 10, wherein means for determining the SNP in codon 388 of FGFR4 gene are means for determining the homozygosity for SNP corresponding to a glycine in codon 388 of FGFR4 (i.e. FGFR4-388Gly allele).

12. A kit according to claim 10 or 11, wherein means for determining the SNP in codon 388 of FGFR4 gene are:

(a) an antibody specifically directed against the polypeptide FGFR4 wherein the amino acid at position 388 is a glycine (FGFR4-388Gly) provided that it cannot bind the polypeptide FGFR4 wherein the amino acid at position 388 is an arginine (FGFR4-388Arg); and/or

(b) an antibody specifically directed against the polypeptide FGFR4 wherein the amino acid at position 388 is an arginine (FGFR4-388Arg) provided that it cannot bind the polypeptide FGFR4 wherein the amino acid at position 388 is a glycine (FGFR4-388Gly); and/or

(c) a pair of primers suitable for amplifying FGFR4 or a fragment of FGFR4 provided that said fragment comprises the SNP in codon 388 of FGFR4; and/or

(d) primers which hybridizes specifically to SNP FGFR4-388Gly or to SNP FGFR4-388Arg.

13. An article of manufacture comprising, or consisting of :

- a packaging material;

- at least one antagonist anti-FGFR4 antibody or a fragment of said antagonist anti-FGFR4 antibody provided that said fragment keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4;

- a label or package insert contained with said packaging material indicating that said at least one antagonist anti-FGFR4 antibody or fragment of said antagonist anti-FGFR4 antibody provided that said fragment keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4 will most likely be effective in treatment of liver cancer in patient suffering from cancer who is homozygous for single nucleotide polymorphism corresponding to the FGFR4-388Gly allele, and whose FGFR4 is activated.

14. Uses according to claims 1, 2, 3 or 7, antagonist of FGFR4 for use according to claim 6, methods according to claims 4, 5, 8 or 9, a kit according to claims 10, 11 or 12 and an article of manufacture according to claim 13, wherein the antagonist FGFR4 antibody is chosen from the group consisting of:

a) an antagonist FGFR4 antibody which comprises:

i) six CDRs having the amino acid sequences SEQ ID Nos: 5, 6, 7, 8, 9, 10; or

ii) six CDRs having the amino acid sequences SEQ ID Nos: 11, 12, 13, 14, 15, 16; or

iii) six CDRs having the amino acid sequences SEQ ID Nos: 17, 18, 19, 20, 21, 22; or

iv) six CDRs having the amino acid sequences SEQ ID Nos: 23, 24, 25, 26, 27, 28; or

v) six CDRs having the amino acid sequences SEQ ID Nos: 29, 30, 31, 32, 33 or 34; or

vi) six CDRs the sequences of which each differ at most by one or two amino acids from the six CDRs of (i), (ii), (iii), (iv) or (v) respectively, provided that the antibody keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4;

and

b) an antagonist FGFR4 antibody which comprises:

i) a CDRH1 comprising or consisting of sequence SEQ ID NO: 71, SEQ ID NO: 5 or SEQ ID NO: 95,

ii) a CDRH2 comprising or consisting of sequence SEQ ID NO: 72, SEQ ID NO: 6 or SEQ ID NO: 96,

- iii) a CDRH3 comprising or consisting of sequence SEQ ID NO: 73 or SEQ ID NO: 7,
- iv) a CDRL1 comprising or consisting of sequence SEQ ID NO: 80 or SEQ ID NO: 94,
- 5 v) a CDRL2 comprising or consisting of sequence SEQ ID NO: 81, and
- vi) a CDRL3 comprising or consisting of sequence SEQ ID NO: 82.

15 **15.** Uses according to claims 1, 2, 3 or 7, antagonist of FGFR4 for use according to claim 6, methods according to claims 4, 5, 8 or 9, a kit according to claims 10, 11 or 12 and article of manufacture according to claim 13, wherein the antagonist FGFR4 antibody comprises a heavy chain variable region and a light chain variable region encoded by nucleotide sequences chosen from the group consisting of:

- (i) SEQ ID Nos. 39 and 41;
- (ii) SEQ ID Nos. 53 and 55;
- 15 (iii) SEQ ID Nos. 57 and 59;
- (iv) SEQ ID Nos. 61 and 63, and
- (v) SEQ ID Nos. 65 and 67;
- (vi) a heavy chain variable region and a light chain variable region encoded by nucleotide sequences at least 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to (i), (ii),
20 (iii), (iv) or (v), provided that the antibody keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4.

25 **16.** Uses according to claims 1, 2, 3 or 7, antagonist of FGFR4 for use according to claim 6, methods according to claims 4, 5, 8 or 9, a kit according to claims 10, 11 or 12 and article of manufacture according to claim 13, wherein the antagonist anti-FGFR4 antibody comprises polypeptide sequences chosen from the group consisting of:

- (i) SEQ ID No. 36 and SEQ ID No. 38; or
- (ii) SEQ ID Nos. 40 and 42; or
- (iii) SEQ ID No. 54 and SEQ ID No. 56; or
- 30 (iv) SEQ ID No. 58 and SEQ ID No. 60; or
- (v) SEQ ID No. 62 and SEQ ID No. 64; or
- (vi) SEQ ID No. 66 and SEQ ID No. 68; or

(vii) SEQ ID Nos.74 and 83; or

(viii) SEQ ID Nos.77 and 85; or

(ix) two polypeptide sequences at least 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to polypeptides of (i), (ii), (iii), (iv), (v), (vi), (vii) or (viii), provided that the antibody

5 keeps its FGFR4 receptor-binding specificity and its antagonist effect on FGFR4.

17. Uses according to claims 1, 2, 3 or 7, antagonist of FGFR4 for use according to claim 6, methods according to claims 4, 5, 8 or 9, wherein FGFR4 is activated when:

10 - the level of phosphorylation of FGFR4, Erk1/2, AKT and/or FRS2 is increased in a biological sample that is a tumor biopsy, compared with a reference level of phosphorylation; and/or

- the level of expression of FGF19 is increased in a biological sample that is a tumor biopsy, cells of tumor micro-environment, blood sample, plasma sample or serum sample, compared with a reference level of expression of FGF19.

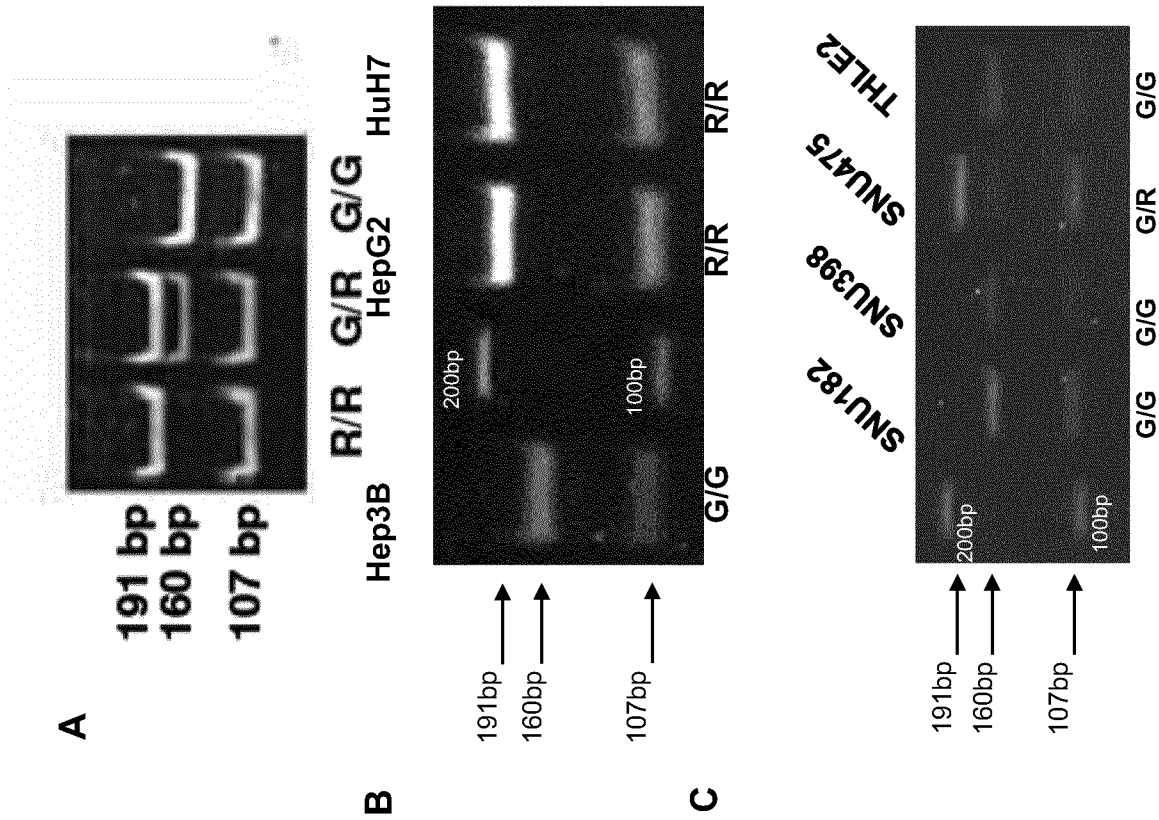


FIG.1

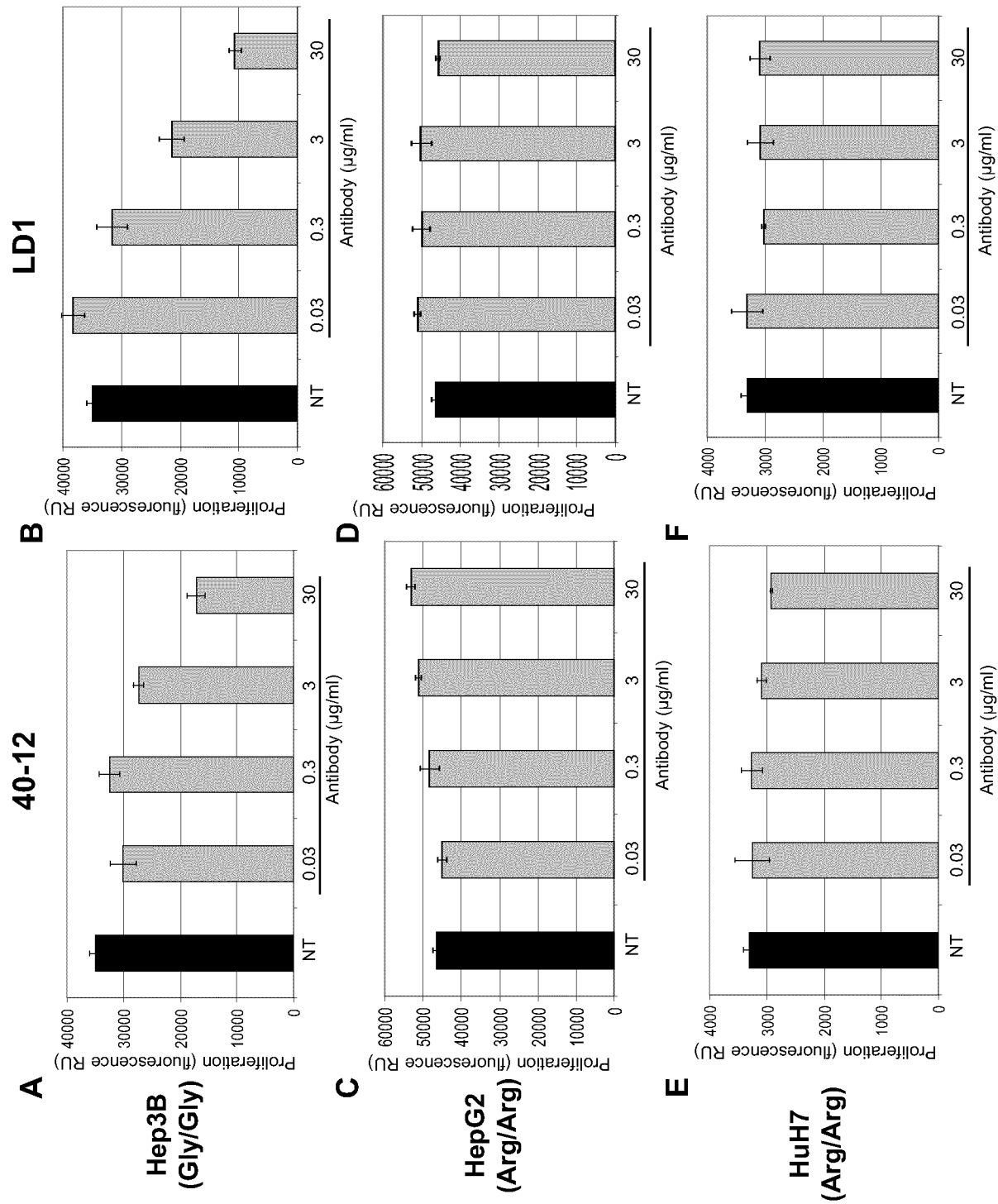


FIG.2

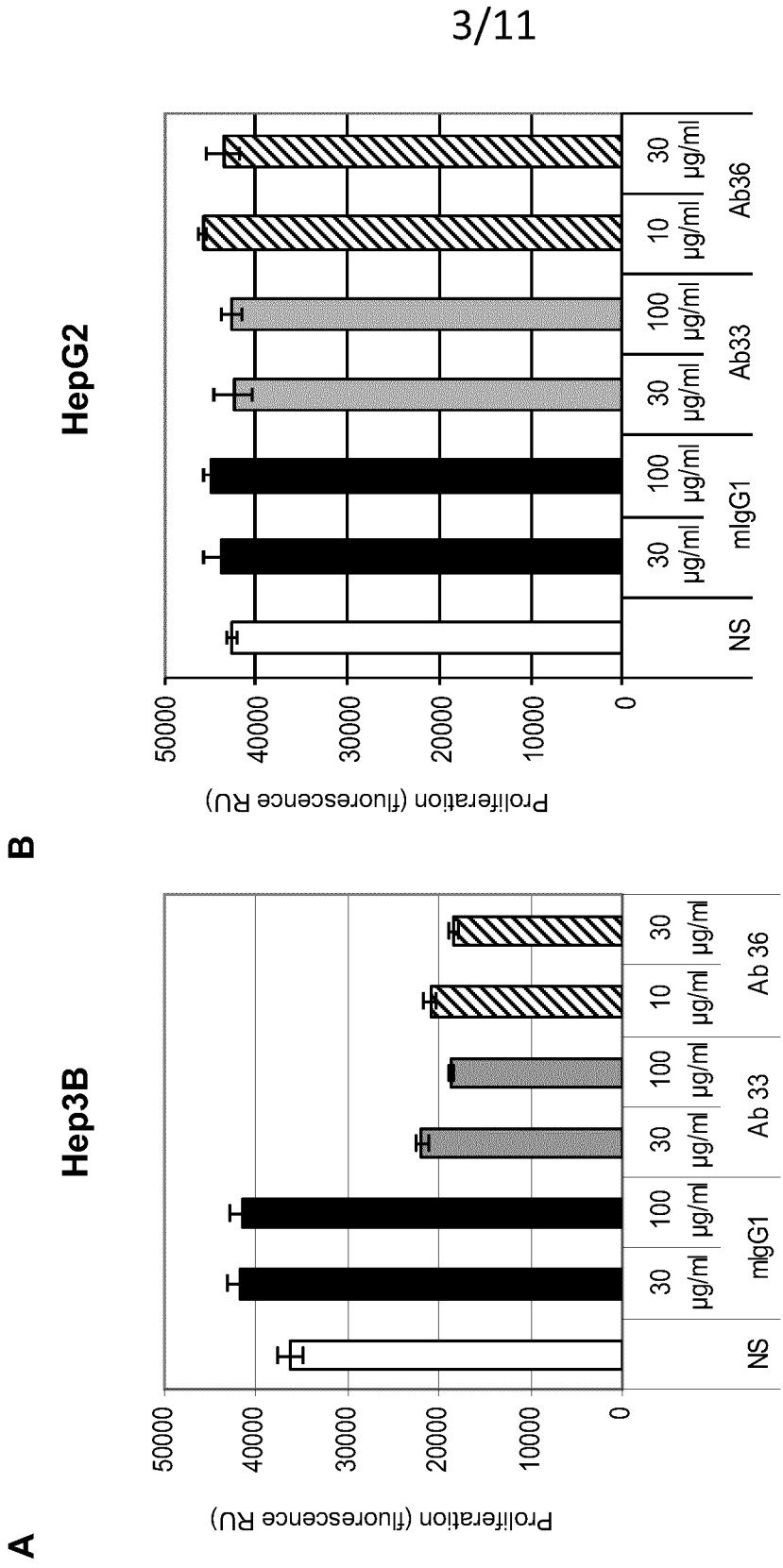


FIG.3

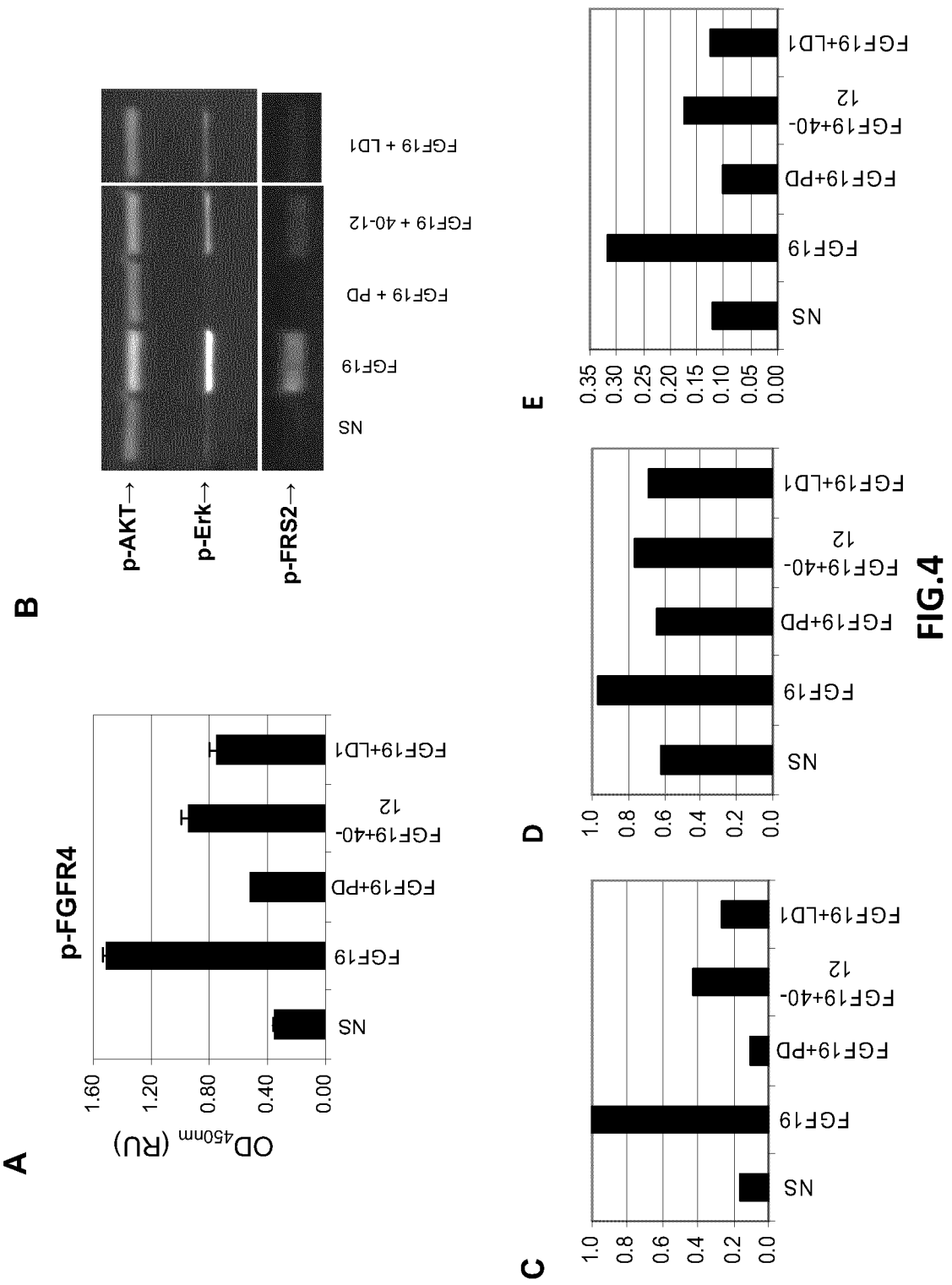


FIG.5

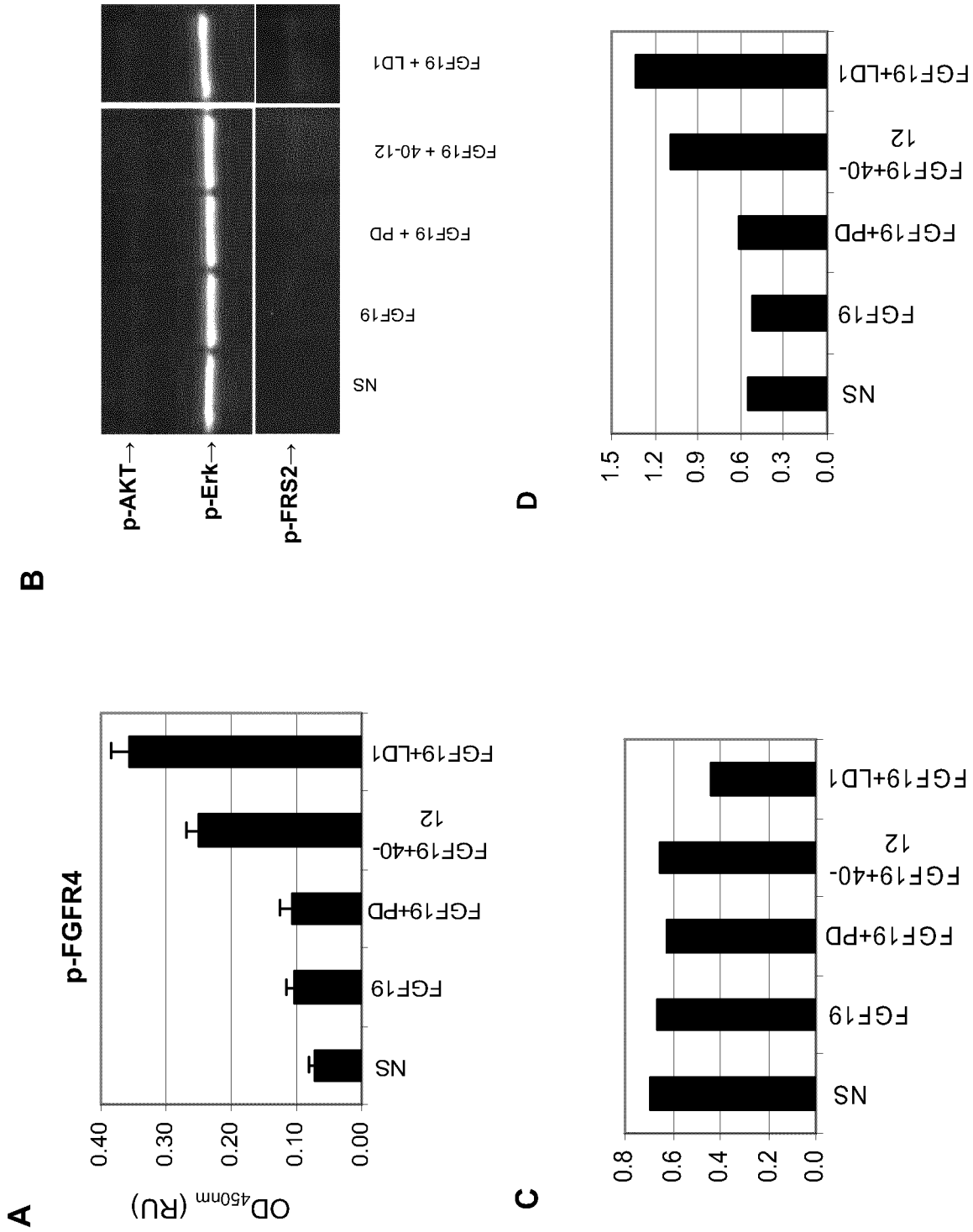
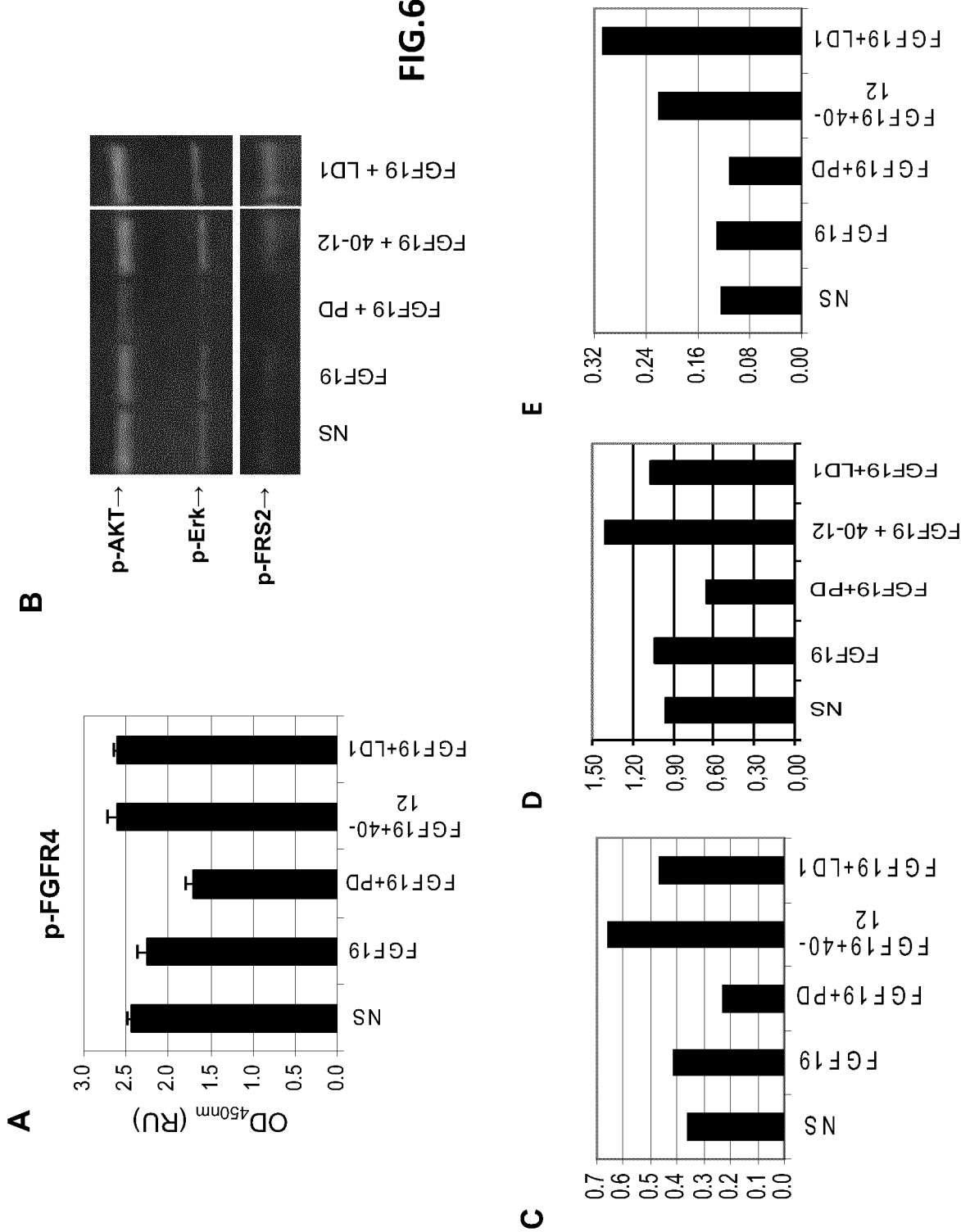
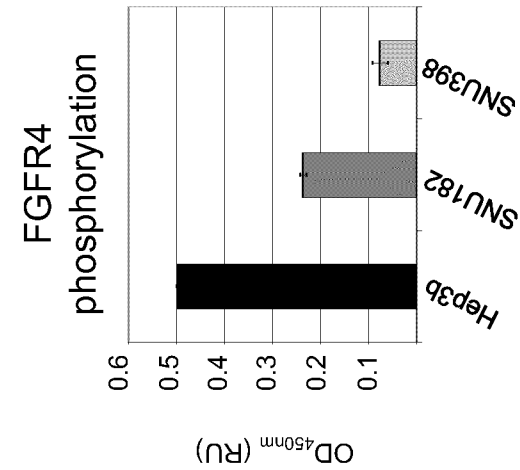


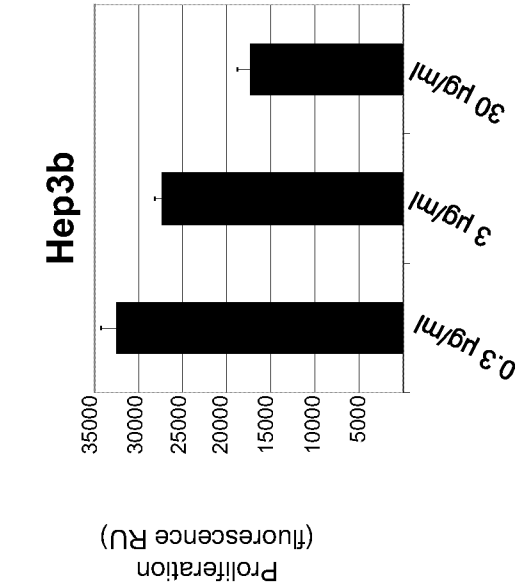
FIG.6



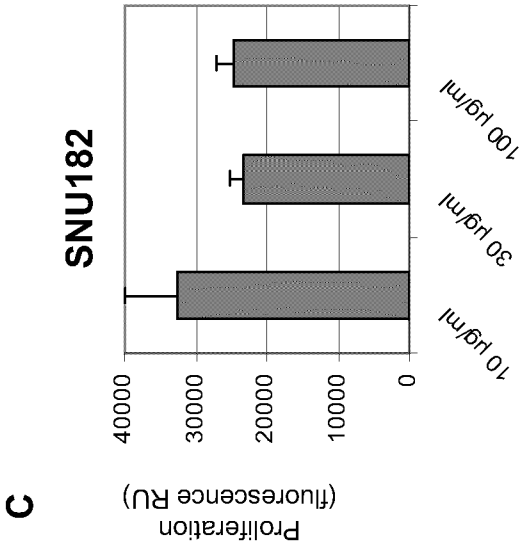
A



B



C



D

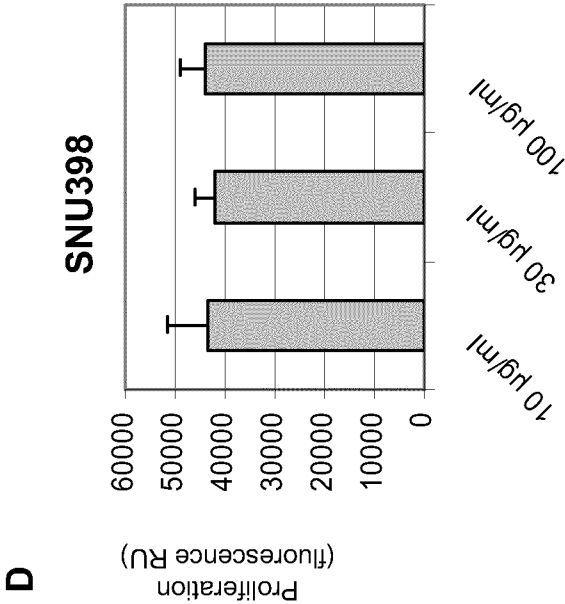
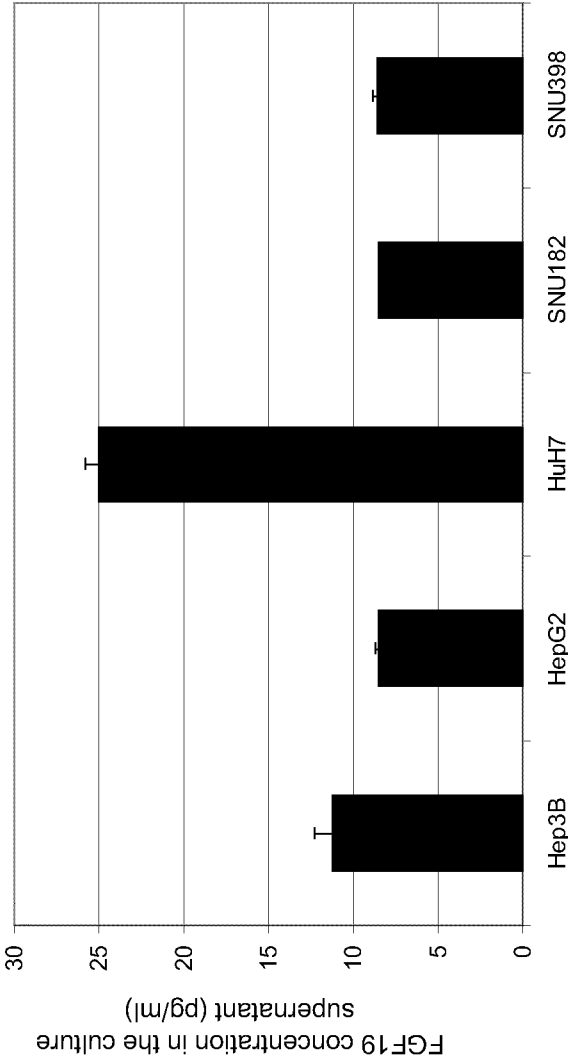


FIG.7

8/11

A



B

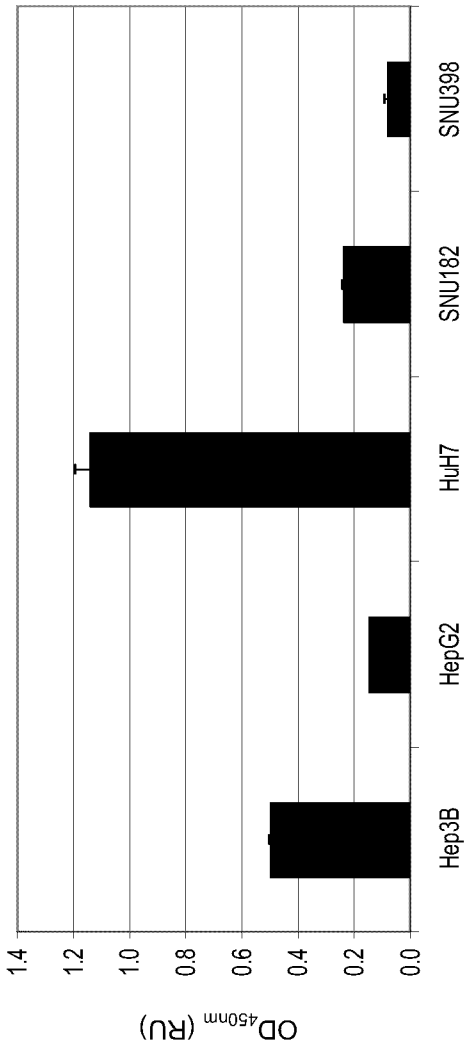
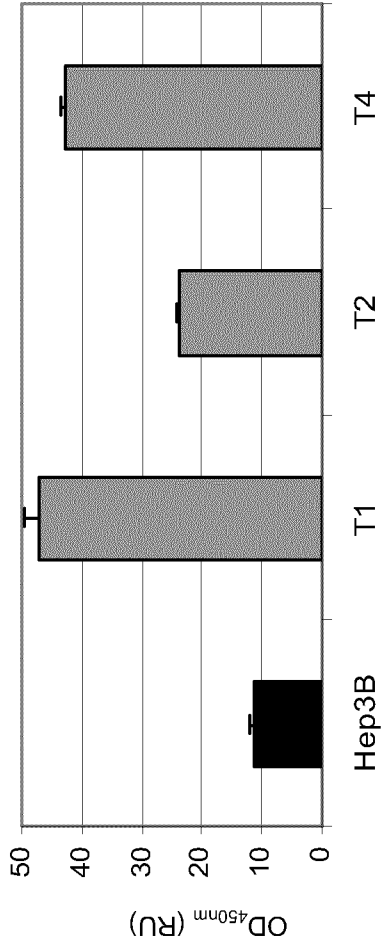


FIG.8

A



B

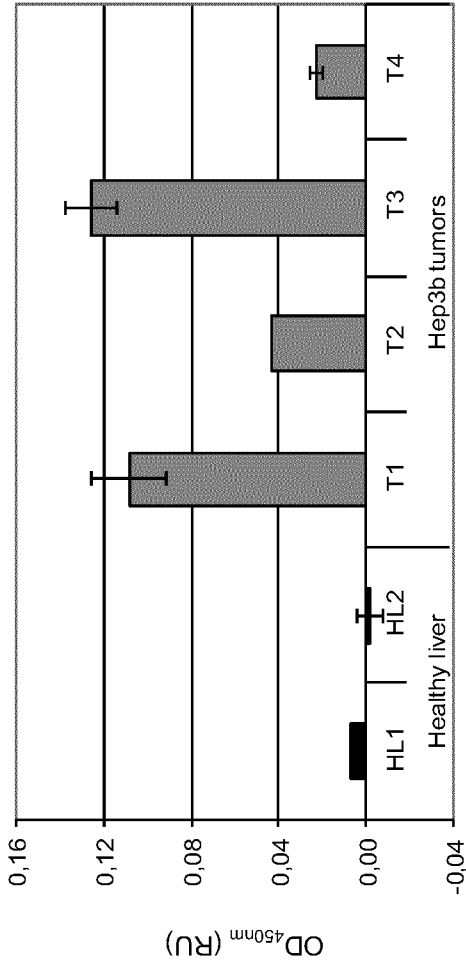


FIG.9

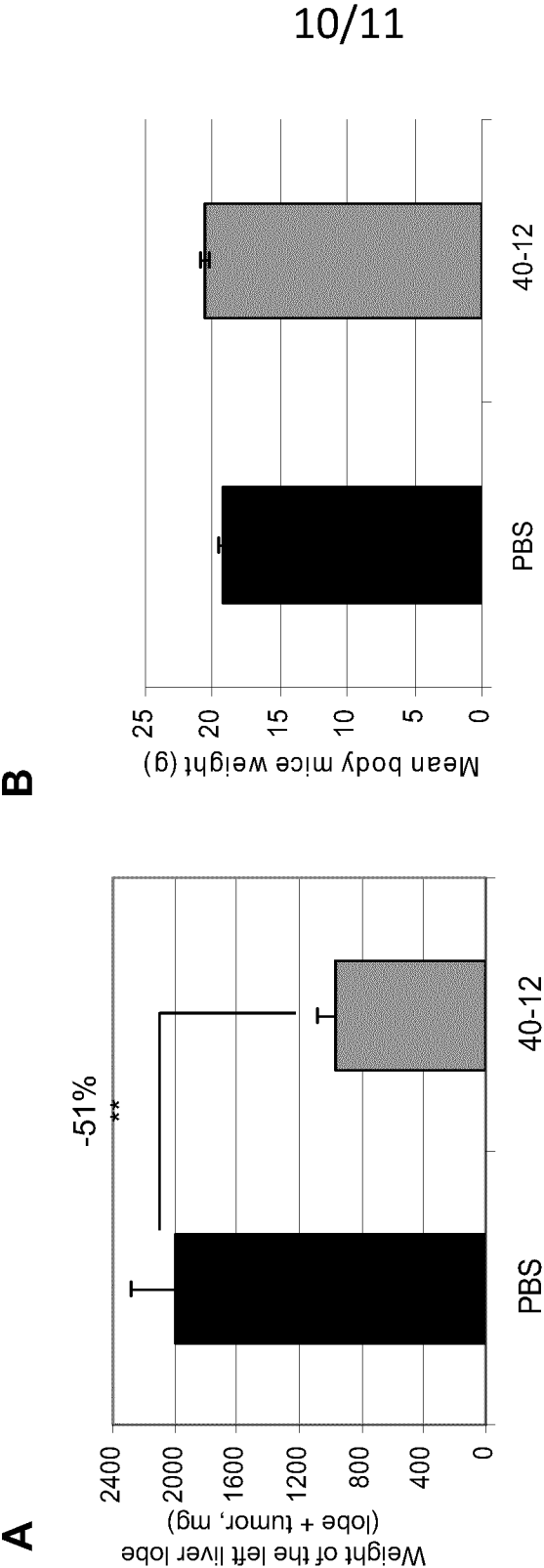


FIG.10

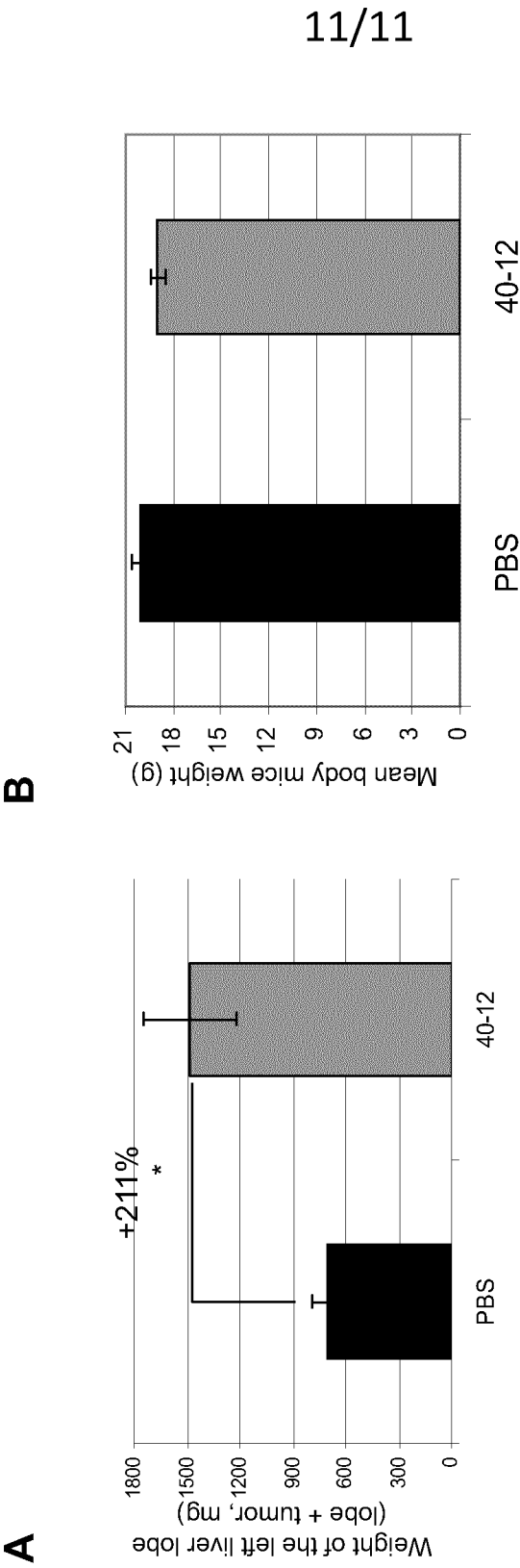


FIG.11

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/050818

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/574
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BANGE J ET AL: "Cancer progression and tumor cell motility are associated with the FGFR4 Arg388 allele", CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 62, 1 February 2002 (2002-02-01), pages 840-847, XP002963936, ISSN: 0008-5472 the whole document In particular: Title; Abstract; Materials and methods section. ----- -/--	1-17



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

17 March 2015

Date of mailing of the international search report

27/03/2015

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Authorized officer

C.F. Angioni

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2015/050818

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HO H K ET AL: "Fibroblast growth factor receptor 4 regulates proliferation, anti-apoptosis and alpha-fetoprotein secretion during hepatocellular carcinoma progression and represents a potential target for therapeutic intervention", JOURNAL OF HEPATOLOGY, ELSEVIER, AMSTERDAM, NL, vol. 50, no. 1, 1 January 2009 (2009-01-01), pages 118-127, XP025769619, ISSN: 0168-8278, DOI: 10.1016/J.JHEP.2008.08.015 [retrieved on 2008-10-12] the whole document In particular: Title; Abstract; Experimental procedures section; Figs. 1, 2 and 5-8. -----	1-17
Y	DOROTHY M. FRENCH ET AL: "Targeting FGFR4 Inhibits Hepatocellular Carcinoma in Preclinical Mouse Models", PLOS ONE, vol. 7, no. 5, 1 January 2012 (2012-01-01), pages e36713-e36713, XP055028245, ISSN: 1932-6203, DOI: 10.1371/journal.pone.0036713 the whole document In particular: Title; Abstract; Materials and methods section. -----	1-17
Y	BUMBACA DANIELA ET AL: "Highly specific off-target binding identified and eliminated during the humanization of an antibody against FGF receptor 4", MABS, LANDES BIOSCIENCE, US, vol. 3, no. 4, 1 July 2011 (2011-07-01), pages 376-386, XP009159783, ISSN: 1942-0870, DOI: 10.4161/MABS.3.4.15786 the whole document In particular: Title; Abstract; Figs. 1-5; Materials and methods section. -----	1-17
Y	US 2010/143386 A1 (ULLRICH AXEL [DE] ET AL) 10 June 2010 (2010-06-10) the whole document In particular: paragraphs 1-11 and 63-97; Figs. 1-7; Claim 4. -----	1-17
Y	US 2013/183319 A1 (BANGE JOHANNES [DE] ET AL) 18 July 2013 (2013-07-18) the whole document In particular: paragraphs 1-9, 48-57 and 128-173; Claims 1-38. -----	1-17
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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2015/050818

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Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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International application No

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