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[Continued on next page]

(54) Title: COMBINATION OF HGF INHIBITOR AND HEDGEHOG INHIBITOR TO TREAT CANCER

(57) Abstract: The present invention is directed toward a method of treating cancer by administering to a patient an inhibitor of Hepatocyte Growth Factor and an inhibitor of the Hedgehog signaling pathway.

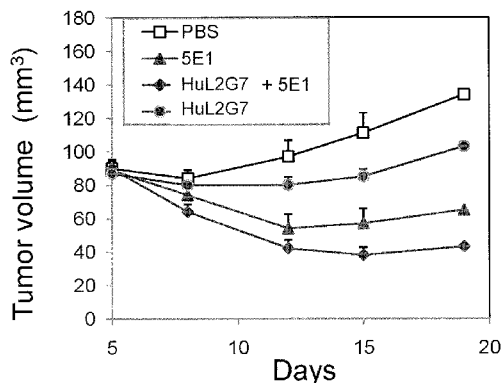


FIGURE 1



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COMBINATION OF HGF INHIBITOR AND HEDGEHOG INHIBITOR TO TREAT CANCER

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit under 35 USC § 119(e) of US Patent Application No. 61/044,444 filed April 11, 2008, which is herewith incorporated in its entirety for all purposes.

STATEMENT OF GOVERNMENT INTEREST

[0002] The invention described in this application was made in part with funding by Grants 5R44 CA101283-03 and 5R01CA108622-04 from the National Institutes of Health. The US Government has certain rights in this invention.

FIELD OF THE INVENTION

[0003] The present invention relates generally to the treatment of cancer, and more particularly, for example, to treatment of cancer with an agent that inhibits Hepatocyte Growth Factor together with an agent that inhibits the Hedgehog signaling pathway.

BACKGROUND OF THE INVENTION

[0004] Human Hepatocyte Growth Factor (HGF) is a multifunctional heterodimeric polypeptide produced by mesenchymal cells. HGF has been shown to stimulate angiogenesis, morphogenesis and motogenesis, as well as the growth and scattering of various cell types (Bussolino *et al.*, J. Cell. Biol. 119: 629, 1992; Zarnegar and Michalopoulos, J. Cell. Biol. 129:1177, 1995; Matsumoto *et al.*, Ciba. Found. Symp. 212:198, 1997; Birchmeier and Gherardi, Trends Cell. Biol. 8:404, 1998; Xin *et al.* Am. J. Pathol. 158:1111, 2001). The pleiotropic activities of HGF are mediated through its receptor, a transmembrane tyrosine kinase encoded by the proto-oncogene cMet. In addition to regulating a variety of normal cellular functions, HGF and its receptor c-Met have been shown to be involved in the initiation, invasion and metastasis of tumors (Jeffers *et al.*, J. Mol. Med. 74:505, 1996; Comoglio and Trusolino, J. Clin. Invest. 109:857, 2002). HGF/cMet are coexpressed, often over-expressed, on various human solid tumors including tumors derived from lung, colon, rectum, stomach, kidney, ovary, skin, multiple myeloma and thyroid tissue (Prat *et al.*, Int. J. Cancer 49:323, 1991; Chan *et al.*, Oncogene 2:593, 1988; Weidner *et al.*, Am. J. Respir. Cell. Mol. Biol. 8:229, 1993; Derksen *et al.*, Blood 99:1405, 2002). HGF acts as an autocrine (Rong *et al.*, Proc. Natl. Acad. Sci. USA 91:4731, 1994; Koochekpour *et al.*, Cancer Res.

57:5391, 1997) and paracrine growth factor (Weidner *et al.*, Am. J. Respir. Cell. Mol. Biol. 8:229, 1993) and anti-apoptotic regulator (Gao *et al.*, J. Biol. Chem. 276:47257, 2001) for these tumors.

[0005] HGF is a 102 kDa protein with sequence and structural similarity to plasminogen and other enzymes of blood coagulation (Nakamura *et al.*, Nature 342:440, 1989; Weidner *et al.*, Am. J. Respir. Cell. Mol. Biol. 8:229, 1993, each of which is incorporated herein by reference). Human HGF is synthesized as a 728 amino acid precursor (preproHGF), which undergoes intracellular cleavage to an inactive, single chain form (proHGF) (Nakamura *et al.*, Nature 342:440, 1989; Rosen *et al.*, J. Cell. Biol. 127:1783, 1994). Upon extracellular secretion, proHGF is cleaved to yield the biologically active disulfide-linked heterodimeric molecule composed of an α -subunit and β -subunit (Nakamura *et al.*, Nature 342:440, 1989; Naldini *et al.*, EMBO J. 11:4825, 1992). The α -subunit contains 440 residues (69 kDa with glycosylation), consisting of the N-terminal hairpin domain and four kringle domains. The β -subunit contains 234 residues (34 kDa) and has a serine protease-like domain, which lacks proteolytic activity. Cleavage of HGF is required for receptor activation, but not for receptor binding (Hartmann *et al.*, Proc. Natl. Acad. Sci. USA 89:11574, 1992; Lokker *et al.*, J. Biol. Chem. 268:17145, 1992). HGF contains 4 putative N-glycosylation sites, 1 in the α -subunit and 3 in the β -subunit. HGF has 2 unique cell specific binding sites: a high affinity ($K_d = 2 \times 10^{-10}$ M) binding site for the cMet receptor and a low affinity ($K_d = 10^{-9}$ M) binding site for heparin sulfate proteoglycans (HSPG), which are present on the cell surface and extracellular matrix (Naldini *et al.*, Oncogene 6:501, 1991; Bardelli *et al.*, J. Biotechnol. 37:109, 1994; Sakata *et al.*, J. Biol. Chem., 272:9457, 1997).

[0006] cMet is a member of the class IV protein tyrosine kinase receptor family. The full length cMet gene was cloned and identified as the cMet proto-oncogene (Cooper *et al.*, Nature 311:29, 1984; Park *et al.*, Proc. Natl. Acad. Sci. USA 84:6379, 1987). The cMet receptor is initially synthesized as a single chain, partially glycosylated precursor, p170^(MET) (Park *et al.*, Proc. Natl. Acad. Sci. USA 84:6379, 1987; Giordano *et al.*, Nature 339:155, 1989; Giordano *et al.*, Oncogene 4:1383, 1989; Bardelli *et al.*, J. Biotechnol. 37:109, 1994). Upon further glycosylation, the protein is proteolytically cleaved into a heterodimeric 190 kDa mature protein (1385 amino acids), consisting of the 50 kDa α -subunit (residues 1-307) and the 145 kDa β -subunit. The cytoplasmic tyrosine kinase domain of the β -subunit is involved in signal transduction.

[0007] Several different approaches have been investigated to obtain HGF inhibitors, i.e. antagonists. Such inhibitors include truncated HGF proteins such as NK1 (N terminal domain plus kringle domain 1; Lokker *et al.*, J. Biol. Chem. 268:17145, 1993); NK2 (N terminal domain plus kringle domains 1 and 2; Chan *et al.*, Science 254:1382, 1991); and NK4 (N-terminal domain plus four kringle domains), which was shown to partially inhibit the primary growth and metastasis of murine lung tumor LLC in a nude mouse model (Kuba *et al.*, Cancer Res. 60:6737, 2000)

[0008] As another approach, Dodge (Master's Thesis, San Francisco State University, 1998) generated antagonist anti-cMet monoclonal antibodies (mAbs). One mAb, 5D5, exhibited strong antagonistic activity in ELISA, but induced a proliferative response of cMet-expressing BAF-3 cells, presumably due to dimerization of the membrane receptors. For this reason, a single-domain form of the anti-cMet mAb 5D5 has been developed as an antagonist (Nguyen *et al.*, Cancer Gene Ther. 10:840, 2003).

[0009] Cao *et al.*, Proc. Natl. Acad. Sci. USA 98:7443, 2001, reported that the administration of a cocktail of three anti-HGF mAbs, which were selected based upon their ability to inhibit the scattering activity of HGF *in vitro*, were able to inhibit the growth of human tumors in the xenograft nude mouse model.

[0010] More recently, several neutralizing (inhibitory) anti-HGF mAbs have been reported including L2G7 (Kim *et al.*, Clin Cancer Res 12:1292, 2006 and US Patent No. 7,220,410), HuL2G7 (WO 07115049 A2), the human mAbs described in WO 2005/017107 A2, and the HGF binding proteins described in WO 07143090 A2 or WO 07143098 A2. It has also been reported that the anti-HGF mAb L2G7, when administered systemically, can strongly inhibit growth or even induce regression of orthotopic (intracranial) glioma xenografts and prolong animal survival (Kim *et al.*, *op. cit.* and WO 06130773 A2).

[0011] The Hedgehog (HH) cellular signaling pathway plays an important role in embryonic development and is also involved in a number of types of cancer (Magliano *et al.*, Nature Reviews Cancer 3:903, 2003; Rubin *et al.*, Nature Reviews Drug Discovery 5:1026, 2006). While the functioning of the HH pathway is complex and not completely understood, it has several components. Three related secreted ligands can activate the pathway: Sonic Hedgehog (SHH), Desert Hedgehog (DHH) and Indian Hedgehog (IHH). These ligands bind to their receptor on the target cell: the Patched 1 (PTCH1) protein, a 12-transmembrane domain (12-TM) protein located at least in part on the plasma membrane. In its unliganded

state, PTCH1 can suppress the activity of Smoothened (SMOH), a 7-TM protein predominantly located in the membrane of intracellular endosomes, by a mechanism that is not entirely clear. However, upon binding HH, PTCH1 can no longer suppress SMOH, ultimately leading to activation of the transcription factors GLI1, GLI2, and GLI3, which in turn upregulate expression of certain genes, thereby stimulating the cell to, e.g., proliferate. A second receptor for the HH ligands, Patched 2 (PTCH2), may act similarly to PTCH1 in certain circumstances (Lee *et al.*, Cancer Res. 66:6964, 2006), and yet other signaling molecules including Suppressor of Fused (SuFu) and Iguana also play a role in the HH signaling pathway.

[0012] The HH pathway has been reported to be involved in a variety of cancers (Magliano *et al.*, *op. cit.*; Rubin *et al.*, *op. cit.*), especially the brain tumor medulloblastoma (Taylor *et al.*, Nat. Genet. 31:306, 2002; Berman *et al.*, Science 297:1559, 2002); the skin cancer basal cell carcinoma (Dahmane *et al.*, Nature 389:876, 1997); small cell lung cancer (Watkins *et al.*, Nature 422:313, 2003); pancreatic cancer (Thayer *et al.*, Nature 425:851, 2003); prostate cancer (Karhadkar *et al.*, Nature 431:707, 2004; Sanchez *et al.*, Proc. Natl. Acad. Sci. USA 101:12561, 2004; Sheng *et al.*, Mol. Cancer 3:29, 2004); breast cancer (Kubo *et al.*, Cancer Res. 64:6071, 2004); and cancers of the digestive tract including esophageal, stomach, pancreatic and biliary tract (Berman *et al.*, Nature 425:846, 2003). For this reason, a number of inhibitors of the HH pathway have been developed, including cyclopamine and KAAD-cyclopamine (Taipale *et al.*, Nature 406:1005, 2000); SANT1-4 (Chen *et al.*, Proc. Natl. Acad. Sci. USA 99:14071, 2002); Cur61414 (Williams *et al.*, Proc. Natl. Acad. Sci. USA 100:8607, 2003); and HhAntag or HhAntag-691 (Romer *et al.*, Cancer Cell 6:229, 2004; Romer and Curan, Cancer Res. 65:4975, 2005); for many of which the target is SMOH. Monoclonal antibodies that bind to SSH and/or other HH ligands, thereby preventing them from binding to PTCH1 and activating the HH pathway, have also been developed (Ericson *et al.*, Cell 87:661, 1996).

SUMMARY OF THE INVENTION

[0013] The invention provides a method of treating cancer by administering to a patient in need of such treatment a first agent that inhibits Hepatocyte Growth Factor (HGF) in combination with a second agent that inhibits the Hedgehog (HH) cellular signaling pathway. In a preferred embodiment, the first agent is a monoclonal antibody (mAb) that binds to and neutralizes HGF. Chimeric, human and humanized anti-HGF mAbs are especially preferred,

particularly humanized L2G7. The second agent is an inhibitor of the Hedgehog signaling pathway, for example a mAb that binds to one or more of the Hedgehog proteins – Sonic Hedgehog, Indian Hedgehog, and Desert Hedgehog – or to the HH receptor Patched 1, thereby inhibiting binding of the HH protein to Patched 1. The method is especially preferred for treating brain cancers such as medulloblastoma, basal cell carcinoma, small cell lung cancer, prostate cancer, breast cancer, and cancers of the digestive tract.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] Figure 1. Graph of tumor growth vs days after tumor implantation of GB-d1 gallbladder tumor xenografts in mice treated with vehicle control PBS, anti-HGF mAb HuL2G7, anti-SHH mAb 5E1 or a combination of HuL2G7 and 5E1 (One outlier mouse was omitted from the HuL2G7 group).

[0015] Figure 2. Graph (Kaplan-Meier plot) of survival of mice injected with RCAS-HGF and RCAS-HSS on day 0 and treated twice weekly with either anti-HGF mAb L2G7 or control mAb 5G8 (2.5 mg/kg) starting on day 14.

[0016] Figure 3. Amino acid sequences of the entire HuL2G7 heavy chain (A) (SEQ ID NO:1) and light chain (B) (SEQ ID NO:2). The first amino acids of the mature heavy and light chain V regions (i.e., after cleavage of the signal sequences) are double underlined and labeled with the number 1; these amino acids are therefore the first amino acids of the light and heavy chains of the actual HuL2G7 mAb. In the heavy chain, the first amino acids of the CH1, hinge, CH2 and CH3 regions are underlined, and in the light chain, the first amino acid of the C_κ region is underlined.

[0017] Figure 4. Amino acid sequences of the light chain (A) (SEQ ID NO:3) and heavy chain (B) (SEQ ID NO:4) variable regions of the 2.12.1 human monoclonal antibody disclosed in WO 2005/017107 A2, therein designated respectively as Seq ID Nos. 38 and 39. The first amino acids of the mature heavy and light variable regions (i.e., after cleavage of the signal sequences), and thus of the actual 2.12.1 mAb, are double underlined.

[0018] Figure 5: Structures of representative small molecule hedgehog signaling pathway inhibitors.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0019] The invention provides a method of treating cancer by administering to a patient in need of such treatment a first agent that inhibits the activity of Hepatocyte Growth Factor (HGF), i.e., an HGF antagonist or cMet antagonist, in combination with (i.e., together with) a second agent that inhibits the Hedgehog (HH) cellular signaling pathway. In many embodiments, the first agent and/or the second agent is a monoclonal antibody (mAb).

1. Antibodies

[0020] Antibodies are very large, complex molecules (molecular weight of ~150,000 or about 1320 amino acids) with intricate internal structure. A natural antibody molecule contains two identical pairs of polypeptide chains, each pair having one light chain and one heavy chain. Each light chain and heavy chain in turn consists of two regions: a variable (“V”) region involved in binding the target antigen, and a constant (“C”) region that interacts with other components of the immune system. The light and heavy chain variable regions fold up together in 3-dimensional space to form a variable region that binds the antigen (for example, a receptor on the surface of a cell). Within each light or heavy chain variable region, there are three short segments (averaging 10 amino acids in length) called the complementarity determining regions (“CDRs”). The six CDRs in an antibody variable domain (three from the light chain and three from the heavy chain) fold up together in 3-D space to form the actual antibody binding site which locks onto the target antigen. The position and length of the CDRs have been precisely defined. Kabat, E. et al., Sequences of Proteins of Immunological Interest, U.S. Department of Health and Human Services, 1983, 1987. The part of a variable region not contained in the CDRs is called the framework, which forms the environment for the CDRs.

[0021] A monoclonal antibody (mAb) is a single molecular species of antibody and therefore does not encompass polyclonal antibodies produced by injecting an animal (such as a rodent, rabbit or goat) with an antigen, and extracting serum from the animal. A humanized antibody is a genetically engineered monoclonal antibody in which the CDRs from a mouse antibody (“donor antibody”, which can also be rat, hamster or other similar species) are grafted onto a human antibody (“acceptor antibody”). Humanized antibodies can also be made with less than the complete CDRs from a mouse antibody (e.g., Pascalis *et al.*, J. Immunol. 169:3076, 2002). Thus, a humanized antibody is an antibody having CDRs from a donor antibody and variable region frameworks and constant regions from human antibodies.

The light and heavy chain acceptor frameworks may be from the same or different human antibodies and may each be a composite of two or more human antibody frameworks; or alternatively may be a consensus sequence of a set of human frameworks (e.g., a subgroup of human antibodies as defined in Kabat *et al.*, op. cit.), i.e., a sequence having the most commonly occurring amino acid in the set at each position. In addition, in order to retain high binding affinity, at least one of two additional structural elements can be employed. See, US Patent No. 5,530,101 and 5,585,089, each of which is incorporated herein by reference, which provide detailed instructions for construction of humanized antibodies.

[0022] In the first structural element, the framework of the heavy chain variable region of the humanized antibody is chosen to have maximal sequence identity (between 65% and 95%) with the framework of the heavy chain variable region of the donor antibody, by suitably selecting the acceptor antibody from among the many known human antibodies. Sequence identity is determined when antibody sequences being compared are aligned according to the Kabat numbering convention. In the second structural element, in constructing the humanized antibody, selected amino acids in the framework of the human acceptor antibody (outside the CDRs) are replaced with corresponding amino acids from the donor antibody, in accordance with specified rules. Specifically, the amino acids to be replaced in the framework are chosen on the basis of their ability to interact with the CDRs. For example, the replaced amino acids can be adjacent to a CDR in the donor antibody sequence or within 4-6 angstroms of a CDR in the humanized antibody as measured in 3-dimensional space.

[0023] A chimeric antibody is an antibody in which the variable region of a mouse (or other rodent) antibody is combined with the constant region of a human antibody; their construction by means of genetic engineering is well-known. Such antibodies retain the binding specificity of the mouse antibody, while being about two-thirds human. The proportion of nonhuman sequence present in mouse, chimeric and humanized antibodies suggests that the immunogenicity of chimeric antibodies is intermediate between mouse and humanized antibodies. Other types of genetically engineered antibodies that may have reduced immunogenicity relative to mouse antibodies include human antibodies made using phage display methods (Dower *et al.*, WO91/17271; McCafferty *et al.*, WO92/001047; Winter, WO92/20791; and Winter, FEBS Lett. 23:92, 1998, each of which is incorporated herein by reference) or using transgenic animals (Lonberg *et al.*, WO93/12227; Kucherlapati WO91/10741, each of which is incorporated herein by reference).

[0024] As used herein, the term “human-like” antibody refers to a mAb in which a substantial portion of the amino acid sequence of one or both chains (e.g., about 50% or more) originates from human immunoglobulin genes. Hence, human-like antibodies encompass but are not limited to chimeric, humanized and human antibodies. As used herein, a “reduced-immunogenicity” antibody is one expected to have significantly less immunogenicity than a mouse antibody when administered to human patients. Such antibodies encompass chimeric, humanized and human antibodies as well as antibodies made by replacing specific amino acids in mouse antibodies that may contribute to B- or T-cell epitopes, for example exposed residues (Padlan, *Mol. Immunol.* 28:489, 1991). As used herein, a “genetically engineered” antibody is one for which the genes have been constructed or put in an unnatural environment (e.g., human genes in a mouse or on a bacteriophage) with the help of recombinant DNA techniques, and would therefore, e.g., not encompass a mouse mAb made with conventional hybridoma technology.

[0025] The epitope of a mAb is the region of its antigen to which the mAb binds. Two antibodies bind to the same or overlapping epitope if each competitively inhibits (blocks) binding of the other to the antigen. That is, a 1x, 5x, 10x, 20x or 100x excess of one antibody inhibits binding of the other by at least 50% but preferably 75%, 90% or even 99% as measured in a competitive binding assay compared to a control lacking the competing antibody (see, e.g., Junghans *et al.*, *Cancer Res.* 50:1495, 1990, which is incorporated herein by reference). Alternatively, two antibodies have the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

2. Antibodies for Use in the Invention

[0026] A monoclonal antibody (mAb) that binds HGF (i.e., an anti-HGF mAb) is said to neutralize HGF, or be neutralizing, if the binding partially or completely inhibits one or more biological activities of HGF (i.e., when the mAb is used as a single agent). Among the biological properties of HGF that a neutralizing antibody may inhibit are the ability of HGF to bind to its cMet receptor, to cause the scattering of certain cell lines such as Madin-Darby canine kidney (MDCK) cells; to stimulate proliferation of (i.e., be mitogenic for) certain cells including hepatocytes, Mv 1 Lu mink lung epithelial cells, and various human tumor cells; or

to stimulate angiogenesis, for example as measured by stimulation of human vascular endothelial cell (HUVEC) proliferation or tube formation or by induction of blood vessels when applied to the chick embryo chorioallantoic membrane (CAM). Antibodies for use in the invention preferably bind to human HGF, i.e., to the protein encoded by the GenBank sequence with Accession number D90334.

[0027] A neutralizing anti-HGF mAb is preferred for use as the first agent in the invention and, at a concentration of, e.g., 0.01, 0.1, 0.5, 1, 2, 5, 10, 20 or 50 $\mu\text{g/ml}$, inhibits a biological function of HGF (e.g., stimulation of proliferation or scattering) by about at least 50% but preferably 75%, more preferably by 90% or 95% or even 99%, and most preferably approximately 100% (essentially completely) as assayed by methods known in the art. Inhibition is considered complete if the level of activity is within the margin of error for a negative control lacking HGF. Typically, the extent of inhibition is measured when the amount of HGF used is just sufficient to fully stimulate the biological activity, or is 0.05, 0.1, 0.5, 1, 3 or 10 $\mu\text{g/ml}$. Preferably, at least 50%, 75%, 90%, or 95% or essentially complete inhibition is achieved when the molar ratio of antibody to HGF is 0.5x, 1x, 2x, 3x, 5x or 10x. Preferably, the mAb is neutralizing, i.e., inhibits the biological activity, when used as a single agent, but optionally 2 mAbs can be used together to give inhibition. Most preferably, the mAb neutralizes not just one but several of the biological activities listed above; for purposes herein, an anti-HGF mAb that used as a single agent neutralizes all the biological activities of HGF is called “fully neutralizing”, and such mAbs are most preferable. Anti-HGF mAbs for use in the invention are preferably specific for HGF, that is they do not bind, or only bind to a much lesser extent (e.g., K_a at least ten-fold less), proteins that are related to HGF such as fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF). Preferred antibodies lack agonistic activity toward HGF. That is, the antibodies block interaction of HGF with cMet without stimulating cells bearing HGF directly. Anti-HGF mAbs for use in the invention typically have a binding affinity (K_a) for HGF of at least 10^7 M^{-1} but preferably 10^8 M^{-1} or higher, and most preferably 10^9 M^{-1} or higher or even 10^{10} M^{-1} or higher.

[0028] Similarly, mAbs that bind and neutralize one or more of the HH proteins (SHH, IHH and DHH) are preferred for use as the second agent in the invention. A neutralizing anti-HH mAb, at a concentration of, e.g., 0.01, 0.1, 0.5, 1, 2, 5, 10, 20 or 50 $\mu\text{g/ml}$, inhibits a biological function of HH (e.g., stimulation of cell proliferation) by about at least 50% but preferably 75%, more preferably by 90% or 95% or even 99%, and most preferably approximately 100% (essentially completely) as assayed by methods known in the art.

Inhibition is considered complete if the level of activity is within the margin of error for a negative control lacking HH. Typically, the extent of inhibition is measured when the amount of HH used is just sufficient to fully stimulate the biological activity, or is 0.05, 0.1, 0.5, 1, 3 or 10 $\mu\text{g/ml}$. Preferably, at least 50%, 75%, 90%, or 95% or essentially complete inhibition is achieved when the molar ratio of antibody to HH is 0.5x, 1x, 2x, 3x, 5x or 10x. Preferably, the mAb is neutralizing, i.e., inhibits the biological activity, when used as a single agent, but optionally 2 or 3 mAbs can be used together to give inhibition. Most preferably, the mAb neutralizes not just one but several of the biological activities of HH; for purposes herein, an anti-HH mAb that used as a single agent neutralizes all the biological activities of HH is called “fully neutralizing”, and such mAbs are most preferable. Anti-HH mAbs for use in the invention are preferably specific for HH, that is they do not bind, or only bind to a much lesser extent (e.g., K_a at least ten-fold less), other proteins that are related to HH. Anti-HH mAbs for use in the invention typically have a binding affinity (K_a) for HH of at least 10^7 M^{-1} but preferably 10^8 M^{-1} or higher, and most preferably 10^9 M^{-1} or higher or even 10^{10} M^{-1} or higher.

[0029] MAbs for use in the invention include antibodies in their natural tetrameric form (2 light chains and 2 heavy chains) and may be of any of the known isotypes IgG, IgA, IgM, IgD and IgE and their subtypes, i.e., human IgG1, IgG2, IgG3, IgG4 and mouse IgG1, IgG2a, IgG2b, and IgG3. The mAbs are also meant to include fragments of antibodies such as Fv, Fab and F(ab')_2 ; bifunctional hybrid antibodies (e.g., Lanzavecchia *et al.*, Eur. J. Immunol. 17:105, 1987), single-chain antibodies (Huston *et al.*, Proc. Natl. Acad. Sci. USA 85:5879, 1988; Bird *et al.*, Science 242:423, 1988); single-arm antibodies (Nguyen *et al.*, Cancer Gene Ther. 10:840, 2003); and antibodies with altered constant regions (e.g., U.S. Patent No. 5,624,821). The mAbs may be of animal (e.g., mouse, rat, hamster or chicken) origin, or they may be genetically engineered. Rodent mAbs are made by standard methods well-known in the art, comprising multiple immunization with HGF in appropriate adjuvant i.p., i.v., or into the footpad, followed by extraction of spleen or lymph node cells and fusion with a suitable immortalized cell line, and then selection for hybridomas that produce antibody binding to HGF, e.g., see under Examples. Chimeric and humanized mAbs, made by art-known methods mentioned *supra*, are preferred for use in the invention. Human antibodies made, e.g., by phage display or transgenic mice methods are also preferred (see e.g., Dower *et al.*, McCafferty *et al.*, Winter, Lonberg *et al.*, Kucherlapati, *supra*). More generally,

human-like, reduced immunogenicity and genetically engineered antibodies as defined herein are all preferred.

[0030] The neutralizing anti-HGF mAb L2G7 (which is produced by a hybridoma deposited at the American Type Culture Collection under ATCC Number PTA-5162 according to the Budapest treaty) as described in Kim *et al.*, Clin Cancer Res 12:1292, 2006 and US Patent No. 7,220,410 and particularly its chimeric and humanized forms such as HuL2G7, as described in WO 07115049 A2, are especially preferred as the first agent in the invention. Neutralizing mAbs with the same or overlapping epitope as L2G7 and/or that compete with L2G7 for binding to HGF are also preferred. MAb that are 90%, 95% or 99% identical to L2G7 in amino acid sequence, when aligned according to the Kabat numbering convention, at least in the CDRs, and maintain its functional properties, or which differ from it by a small number of functionally inconsequential amino acid substitutions (e.g., conservative substitutions), deletions, or insertions can also be used in the invention.

[0031] Also preferred for use as the first agent in the invention are the anti-HGF mAbs described in WO 2005/017107 A2, whether explicitly by name or sequence or implicitly by description or relation to explicitly described mAbs. Especially preferred mAbs are those produced by the hybridomas designated therein as 1.24.1, 1.29.1, 1.60.1, 1.61.3, 1.74.3, 1.75.1, 2.4.4, 2.12.1, 2.40.1 and 3.10.1, and respectively defined by their heavy and light chain variable region sequences provided by SEQ ID NO's 24 – 43, with 2.12.1 being most preferred; mAbs possessing the same respective CDRs as any of these listed mAbs; mAbs having light and heavy chain variable regions that are at least 90%, 95% or 99% identical to the respective variable regions of these listed mAbs or differing from them only by inconsequential amino acid substitutions, deletion or insertions; mAbs binding to the same epitope of HGF as any of these listed mAbs, and all mAbs encompassed by claims 1 through 94 therein.

[0032] Alternatively, any of the HGF binding proteins described in WO07143090A2 or WO07143098A2 may be used as the first agent in the invention.

[0033] Native mAbs for use in the invention may be produced from their hybridomas. Genetically engineered mAbs, e.g., chimeric or humanized mAbs, may be expressed by a variety of art-known methods. For example, genes encoding their light and heavy chain V regions may be synthesized from overlapping oligonucleotides and inserted together with available C regions into expression vectors (e.g., commercially available from Invitrogen)

that provide the necessary regulatory regions, e.g., promoters, enhancers, poly A sites, etc. Use of the CMV promoter-enhancer is preferred. The expression vectors may then be transfected using various well-known methods such as lipofection or electroporation into a variety of mammalian cell lines such as CHO or non-producing myelomas including Sp2/0 and NS0, and cells expressing the antibodies selected by appropriate antibiotic selection. See, e.g., US Patent No. 5,530,101. Larger amounts of antibody may be produced by growing the cells in commercially available bioreactors.

[0034] Once expressed, the mAbs for use in the invention may be purified according to standard procedures of the art such as microfiltration, ultrafiltration, protein A or G affinity chromatography, size exclusion chromatography, anion exchange chromatography, cation exchange chromatography and/or other forms of affinity chromatography based on organic dyes or the like. Substantially pure antibodies of at least about 90 or 95% homogeneity are preferred, and 98% or 99% or more homogeneity most preferred, for pharmaceutical uses. The mAbs are typically provided in a pharmaceutical formulation, i.e., in a physiologically acceptable carrier, optionally with excipients or stabilizers. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, or acetate at a pH typically of 5.0 to 8.0, most often 6.0 to 7.0; salts such as sodium chloride, potassium chloride, etc. to make isotonic; antioxidants, preservatives, low molecular weight polypeptides, proteins, hydrophilic polymers such as polysorbate 80, amino acids, carbohydrates, chelating agents, sugars, and other standard ingredients known to those skilled in the art (Remington's Pharmaceutical Science 16th edition, Osol, A. Ed. 1980). The mAb is typically present at a concentration of 1 - 100 mg/ml, e.g., 10 mg/ml.

3. Other Agents for Use in the Invention

[0035] Besides anti-HGF mAbs, the first agent for use in the invention may be any other agent that inhibits HGF, i.e., inhibits its biological activity, and may therefore be called an HGF antagonist. Examples are soluble forms of cMet (e.g., see Michieli *et al.*, Cancer Cell 6:61, 2004) and a cocktail of several anti-HGF mAbs (Cao *et al.*, Proc. Natl. Acad. Sci. USA 98:7443, 2001). As used herein the term "agent that inhibits HGF" or "HGF inhibitor" also includes an agent that interacts with the cMet receptor of HGF so as to inhibit HGF signaling through cMet; such an agent may also be called a cMet inhibitor or antagonist. However, as used herein, inhibitors or antagonists of HGF or cMet or the HGF/cMet pathway are not

meant to include agents that inhibit signaling events, such as activation of MAP kinase, that occur after (i.e., downstream) of the HGF-cMet interaction and activation of cMet, and which the HGF/cMet pathway shares with other ligand/receptor systems. A cMet antagonist may function by binding to cMet and competitively blocking binding of HGF or activation by HGF. Exemplary agents include truncated HGF proteins such as NK1, NK2, and NK4 (*supra*) and anti-cMet mAbs. A preferred example is an anti-cMet antibody that has been genetically engineered to have only one “arm”, i.e. binding domain, such as OA-5D5 (Martens *et al.*, Clin. Cancer Res. 12:6144, 2006). Such agents may also be small molecule inhibitors of the tyrosine kinase activity of cMet including SU5416 (Wang *et al.*, J Hepatology 41:267, 2004), and ARQ 197 being developed by ArQule, Inc. (Abstract Number 3525 at the 2007 Annual Meeting of the American Society of Clinical Oncology), which may be administered orally.

[0036] The second agent for use in the invention is any inhibitor of the Hedgehog (HH) signaling pathway (particularly the HH pathway in humans), e.g., an agent that inhibits the ability of an HH protein to stimulate a cell via this pathway, also called an HH pathway inhibitor or simply HH inhibitor. Such an agent may bind to the one or more of the HH ligands – Sonic Hedgehog (SHH), Indian Hedgehog (IHH) and Desert Hedgehog (DHH) – or to their Patched 1 (PTCH1) or Patched 2 (PTCH2) receptors or to a downstream mediator such as Smoothened (SMOH) or SuFu or Iguana (also known as DZIP1), or to one or more of the transcription factors GLI1, GLI2, and GLI3 activated by the pathway. All of these hedgehog pathway proteins are well known human proteins for which sequences are available from UniProtKB/Swiss-Prot and similar databases. Insofar as a protein has more than one known form in a species due to natural allelic variation between individuals, an inhibitor can bind to and inhibit any, or all, of such known allelic forms, and preferably binds to and inhibits the wildtype, most common or first published allelic form. Exemplary sequences for human SHH, IHH and DHH are assigned UniProtKB/Swiss-Prot accession numbers Q15465, Q14623, O43323 and respectively. Exemplary sequences for other human hedgehog pathway proteins are: PTCH1 (Q13635), PTCH2 (Q9Y6C5), SMOH (Q99835), DZIP1 (Q86YF9), SuFu (Q9UMX1), Gli1 (P08151), Gli2 (P10070), Gli3 (P10071). The agent may be a protein such as a mAb, preferably a chimeric, humanized or human mAb, e.g., humanized or chimeric 5E1, which binds to one or more of the HH proteins or to PTCH1 (or PTCH2), or may be a small molecule (i.e., a compound having relatively low molecular weight, most often less than 500 or 600 or 1000 kDa). Exemplary small molecule

second agents are cyclopamine, KAAD-cyclopamine (3-Keto-N-(aminoethyl-aminocaproyl-dihydrocinnamoyl)cyclopamine), SANT1-4 (Chen et al., Proc. Natl. Acad.Sci. USA 2002, 99: 14071 –14076, see Fig. 3A), CUR61414 (see Fig. 1A of Williams et al., PNAS 2003 100: 8 4616-4621) and HhAntag-691 (Romer et al., Cancer Cell. 2004; 6:229–240); JK814 Lee, ChemBioChem 2007, 8: 1916–1919 (Fig. 1A) (all incorporated by reference). Other examples of HH antagonists are described by WO/2004/020599 and Katoh, Cancer Biol Ther. 2005 4:1050-4, and US 7,300,929 (all incorporated by reference). The structures of several of the small molecule inhibitors are shown in Fig. 5 herein. Proteins are typically administered parenterally, e.g. intravenously, whereas small molecules may be administered parenterally or orally.

4. Treatment Methods

[0037] The invention provides methods of treatment in which the indicated first and second agents are administered to patients having a cancer (therapeutic treatment) or at risk of occurrence or recurrence of cancer (prophylactic treatment). The term “patient” includes human patients; veterinary patients, such as cats, dogs and horses; farm animals, such as cattle, sheep, and pigs; and laboratory animals used for testing purposes, such as mice and rats. The methods are particularly amenable to treatment of human patients. The mAb or other agent used in methods of treating human patients binds to the respective human protein. A mAb or other agent to a human protein can also be used in other species in which the species homolog has antigenic crossreactivity with the human protein. In species lacking such crossreactivity, an antibody or other agent is used with appropriate specificity for the species homolog present in that species. However, in xenograft experiments in laboratory animals, a mAb with specificity for the human protein expressed by the xenograft is generally used.

[0038] A mAb or other protein used as a first or second agent in the methods of the invention can be administered to a patient by any suitable route, especially parentally by intravenous (IV) infusion or bolus injection, intramuscularly or subcutaneously or intraperitoneally. IV infusion can be given over as little as 15 minutes, but more often for 30 minutes, 60 minutes, 90 minutes or even 2 or 3 hours. The agent can also be injected directly into the site of disease (e.g., the tumor itself; or the brain or its surrounding membranes or cerebrospinal fluid in the case of a brain tumor) or encapsulated into carrying agents such as liposomes. However, when treating brain tumors (i.e., a tumor existing within the brain of

the patient), systemic administration of the mAb, e.g., by IV infusion, is possible and even preferred (see WO 06130773 A2). The dose given to a patient having a cancer is sufficient to alleviate or at least partially arrest the disease being treated (“therapeutically effective dose”) and is sometimes 0.1 to 5 mg/kg body weight, for example 1, 2, 3, 4, 5 or 6 mg/kg, but may be as high as 10 mg/kg or even 15 or 20 or 30 mg/kg. A fixed unit dose may also be given, for example, 50, 100, 200, 500 or 1000 mg, or the dose may be based on the patient’s surface area, e.g., 100 mg/m². Usually between 1 and 8 doses, (e.g., 1, 2, 3, 4, 5, 6, 7 or 8) are administered to treat cancer, but 10, 12, 20 or more doses may be given. The agent can be administered daily, biweekly, weekly, every other week, monthly or at some other interval, depending, e.g. on its half-life, for 1 week, 2 weeks, 4 weeks, 8 weeks, 3-6 months or longer, or until the disease progresses. Repeated courses of treatment are also possible, as is chronic administration.

[0039] When a small molecule is used as the first or second agent, it is typically administered more often, preferably once a day, but 2, 3, 4 or more times per day is also possible, as is every two days, weekly or at some other interval. Small molecule drugs are often taken orally but parenteral administration is also possible, e.g., by IV infusion or bolus injection or subcutaneously or intramuscularly. Doses of small molecule drugs are typically 10 to 1000 mg, with 100, 150, 200 or 250 mg very typical, with the optimal dose established in clinical trials. For either a protein or small molecule drug, a regime of a dosage and intervals of administration that alleviates or at least partially arrests the symptoms of a disease (biochemical, histologic and/or clinical), including its complications and intermediate pathological phenotypes in development of the disease is referred to as a therapeutically effective regime.

[0040] When a first agent (an HGF inhibitor) is used in combination with a second agent (an HH pathway inhibitor), the combination may take place over any convenient timeframe. For example, each agent may be administered to a patient on the same day, and the agents may even be administered in the same intravenous infusion. However, the agents may also be administered on alternating days or alternating weeks, fortnights or months, and so on. In some methods, the respective agents are administered with sufficient proximity in time that the agents are simultaneously present (e.g., in the serum) at detectable levels in the patient being treated. In some methods, an entire course of treatment of one agent consisting of a number of doses over a time period (see above) is followed by a course of treatment of the other agent also consisting of a number of doses. In some methods, treatment with the agent

administered second is begun if the patient has resistance or develops resistance to the agent administered initially. The patient may receive only a single course of treatment with each agent or multiple courses with one or both agents. Frequently, a recovery period of 1, 2 or several days or weeks is allowed between administration of the two agents if this is beneficial to the patient in the judgment of the attending physician. When a suitable treatment regimen has already been established for one of the agents, that regimen is preferably used when the agent is used in combination with the other. Typically, these agents are administered until the disease progresses.

[0041] Optionally, an HGF and a hedgehog inhibitor can be combined in a kit, for example, as separate vials in the same package, or holder. The kit can contain instructions for performing any of the methods described herein. Some combinations of a HGF inhibitor and a hedgehog inhibitor (for example, two antibodies), can also be mixed in the same composition. Such compositions and kits can be formed either by a manufacturer or by a health care provider.

[0042] The methods of the invention can also be used in prophylaxis of a patient at risk of cancer. Such patients include those having genetic susceptibility to cancer, patients who have undergone exposure to carcinogenic agents, such as radiation or toxins, and patients who have undergone previous treatment for cancer and are at risk of recurrence. A prophylactic dosage is an amount sufficient to eliminate or reduce the risk, lessen the severity, or delay the outset of the disease, including biochemical, histologic and/or clinical symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. Administration of a pharmaceutical composition in an amount and at intervals effective to effect one or more of these objects is referred to as a prophylactically effective regime. The dosages and regimens disclosed above for therapeutic treatment can also be used for prophylactic treatment.

[0043] Types of cancer especially susceptible to treatment using the methods of the invention include solid tumors known or suspected to require angiogenesis or to be associated with elevated levels of HGF or cMet (which can be measured at the mRNA or protein level relative to noncancerous tissue of the same type, optionally from the same patient), for example ovarian cancer, breast cancer, lung cancer (small cell or non-small cell), colon cancer, prostate cancer, pancreatic cancer, bladder cancer, cervical cancer, renal cancer, gastric cancer, liver cancer, head and neck tumors, mesothelioma, melanoma, and sarcomas,

and brain tumors. Treatment can also be administered to patients having leukemias or lymphomas. The methods of the invention are particularly suitable for treatment of brain tumors including meningiomas; gliomas including ependymomas, oligodendrogliomas, and all types of astrocytomas (low grade, anaplastic, and glioblastoma multiforme or simply glioblastoma); gangliogliomas, schwannomas, chordomas; and brain tumors primarily of children, particularly medulloblastoma but also including primitive neuroectodermal tumors. Both primary brain tumors (i.e., arising in the brain) and secondary or metastatic brain tumors can be treated by the methods of the invention. Tumors associated with activation of the HH pathway such as basal cell carcinoma, medulloblastoma, small cell lung cancer, prostate cancer, breast cancer, and cancers of the digestive tract including esophageal, stomach, pancreatic and biliary tract are also especially susceptible to treatment by the methods of the invention.

[0044] Because of the severity of cancer, several drugs to treat the disease are often given in combination. Hence, in a preferred embodiment of the present invention, the first agent (an HGF inhibitor) and the second agent (an HH pathway inhibitor) are administered together with additional anti-cancer drugs. The first agent and second agent can be administered before, during or after the other anti-cancer drugs. For example, the first and second agents may be administered together with any one or more of the chemotherapeutic drugs known to those of skill in the art of oncology, for example alkylating agents such as carmustine, chlorambucil, cisplatin, carboplatin, oxaliplatin, procarbazine, and cyclophosphamide; antimetabolites such as fluorouracil, floxuridine, fludarabine, gemcitabine, methotrexate and hydroxyurea; natural products including plant alkaloids and antibiotics such as bleomycin, doxorubicin, daunorubicin, idarubicin, etoposide, mitomycin, mitoxantrone, vinblastine, vincristine, and Taxol (paclitaxel) or related compounds such as Taxotere®; the topoisomerase 1 inhibitor irinotecan; agents specifically approved for brain tumors including temozolomide and Gliadel® wafer containing carmustine; and inhibitors of tyrosine kinases such as Gleevec®, Sutent® (sunitinib malate) and Tarceva® (erlotinib); and all approved and experimental anti-cancer agents listed in WO 2005/017107 A2 (which is herein incorporated by reference). The first and second agents can be administered in combination with 1, 2, 3 or more of these other agents used in a standard chemotherapeutic regimen. Normally, the other agents are those already known to be effective for the particular type of cancer being treated. Moreover, the first and second agents can be administered together with any form of radiation therapy including external beam radiation,

intensity modulated radiation therapy (IMRT) and any form of radiosurgery including Gamma Knife, Cyberknife, Linac, and interstitial radiation (e.g. implanted radioactive seeds, GliSite balloon), and/or with surgery. Combination with radiation therapy can be especially appropriate for head and neck cancer and brain tumors. Other agents with which the first and second agents can be administered include biologics such as monoclonal antibodies, including Herceptin™ against the HER2 antigen, Avastin™ against VEGF, and Erbitux® (cetuximab) and Vectibix® (panitumumab) against the Epidermal Growth Factor (EGF) receptor (EGFR).

[0045] The progression-free survival or overall survival time of patients with cancer (e.g., ovarian, prostate, breast, lung, colon, pancreatic, kidney, and brain, especially when relapsed or refractory) treated according to the method of the invention with the first and second agents may increase by at least 10%, 20%, 30% or 40% but preferably 50%, 60% to 70% or even 80%, 90%, 100% or longer, compared to patients treated similarly (e.g., with standard chemotherapy or without specific therapy) but without the first and second agents. The median progression-free survival or overall survival time may also be increased by at least 10 days, but preferably 30 days, 60 days, or 3, 4, 5 or 6 months or 1 year or longer by treatment according to the method of the invention. In addition or alternatively, treatment by the method of the invention may increase the complete response rate, partial response rate, or objective response rate (complete + partial) of patients by at least 10%, 20%, 30% or 40% but preferably 50%, 60% to 70% or even 80%, 90% or 100%. Moreover, when administering treatment with two agents, the regimes with which the respective agents are administered are combined in such a manner that each agent can make a contribution to the therapy, so treatment according to the invention with the first and second agents can increase progression-free or overall survival or increase the complete, partial or objective response rate by at least 10%, 20%, 30% or 40% but preferably 50%, 60% to 70% or even 80%, 90% or 100% compared to treatment with either agent without the other. Indeed, preferably treatment with the first and second agents is synergistic, i.e., better than additive. Optionally, treatment according to the method of the invention can inhibit tumor invasion, or metastasis.

[0046] Typically, in a clinical trial (e.g., a phase II, phase II/III or phase III trial), the aforementioned increases in median progression-free survival and/or response rate of the patients treated by the method of the invention together with a standard therapy (e.g., a chemotherapeutic regimen), relative to the control group of patients receiving the standard therapy alone, is statistically significant, for example at the $p < 0.05$ or 0.01 or even 0.001

level. The complete and partial response rates can be determined by objective criteria commonly used in clinical trials for cancer, e.g., as listed or accepted by the National Cancer Institute and/or Food and Drug Administration.

EXAMPLES

1. HGF and HH inhibitors in xenograft models

[0047] The ability of treatment with a first agent that inhibits the activity of HGF (i.e., an HGF antagonist or cMet antagonist), in combination with a second agent that inhibits the HH signaling pathway, to inhibit growth of human tumors is demonstrated in xenograft models in immunodeficient mice or other rodents such as rat. Illustrative but not limiting examples of immunodeficient strains of mice that can be used are nude mice such as CD-1 nude, Nu/Nu, Balb/c nude, NIH-III (NIH-bg-nu-xid BR); scid mice such as Fox Chase SCID (C.B-17 SCID), Fox Chase outbred SCID and SCID Beige; mice deficient in RAG enzyme; as well as nude rats. Experiments are carried out as described previously (Kim *et al.*, Nature 362:841, 1992, which is incorporated herein by reference). Human tumor cells typically grown in complete DMEM medium are typically harvested in HBSS. Female immunodeficient, e.g., athymic nude mice (4-6 wks old) are injected s.c. with typically 5×10^6 cells in 0.2 ml of HBSS in the dorsal areas. When the tumor size reaches 50-100 mm³, the mice are grouped randomly and appropriate amounts of the agents are administered. For example, an anti-HGF or other mAb (typically between 0.1 and 1.0 mg, e.g. 0.5 mg) is administered i.p. once, twice or three times per week in a volume of, e.g., 0.1 ml, for e.g., 1, 2, 3, or 4 weeks or the duration of the experiment. An orally active small molecule agent may be administered in drinking water or by injection. Tumor sizes are determined typically twice a week by measuring in two dimensions [length (a) and width (b)]. Tumor volume is calculated according to $V = ab^2/2$ and expressed as mean tumor volume $\pm$ SEM. The number of mice in each treatment group is at least 3, but more often between 5 and 10, e.g., 7. One group of mice is treated with both agents; other groups may be treated with neither agent or with one agent but not the other agent. Omitted agents may optionally be substituted by a "placebo" of like kind, e.g., an irrelevant mAb instead of an active mAb. Statistical analysis may be performed using, e.g., Student's t test. In a variation of this experiment, administration of the agents begins simultaneously or shortly after injection of the tumor cells. The effect of the agents may be measured by growth of the tumor with time, prolongation of the survival of the mice, or increase in percent of the mice surviving at a given time or indefinitely.

[0048] Various tumor cell lines known to secrete or respond to HGF are used in separate experiments, for example U87 or U118 human glioblastoma cells, and/or GB-d1 human gallbladder tumor cells. Preferably, the cells also secrete and/or respond to one or more HH proteins. Preferred mAbs to be used as the first agent are neutralizing anti-HGF mAbs that are human-like and/or have reduced-immunogenicity, such as the L2G7 mAb and its chimeric and humanized forms and mAbs with the same epitope as L2G7. Preferred second agents are cyclopamine and mAbs that bind and neutralize one of more of the HH proteins, e.g., mAb 5E1 (Ericson et al., Cell 87:661, 1996), available from University of Iowa hybridoma bank. The combination of first and second agents inhibits the growth of tumor xenografts by at least 25%, but possibly 40% or 50%, and as much as 75% or 90% or greater, or even completely inhibits tumor growth after some period of time or causes tumor regression or disappearance. There may also be this extent of increased inhibition when both agents are used compared to only one. This inhibition takes place for at least tumor cell lines such as U87 or U118 in at least one mouse strain such as NIH III Beige/Nude, but preferably occurs for 2, 3, several, many, or even essentially all HGF-expressing tumor cell lines of a particular (e.g., glioma) or any type, when tested in one or more immunodeficient mouse strains that do not generate a neutralizing antibody response against the injected antibody. Treatment with some combinations of first and second agents in one or more of the xenograft models leads to the indefinite survival of 50%, 75%, 90% or even essentially all mice, who would otherwise die or need to be sacrificed because of growth of their tumor.

[0049] For example, such an experiment was performed with GB-d1 gallbladder tumor xenografts. Female NIH III xid/Beige/nude mice (4-6 wks old) were implanted with tumors by s.c. injection of 10^6 GB-d1 cells in the dorsal areas. When the tumor size reached $\sim 100 \text{ mm}^3$, the mice were grouped randomly into 4 groups of 5 mice each. Mice in the respective groups received either PBS; HuL2G7 anti-HGF mAb; 5E1 anti-SHH mAb (Ericson et al., *op. cit.*) or a combination (i.e., both) of HuL2G7 and 5E1. The mAbs were administered twice per week at 100 μg (approx. 5 mg/kg body weight) from day 5. Tumor sizes were determined twice per week as described above. Fig. 1 shows that while treatment with either L2G7 or 5E1 partially inhibited tumor growth, the combination of mAbs inhibited tumor growth more strongly than either agent alone.

[0050] Similar tumor inhibition experiments are performed with the HGF inhibitor (e.g., L2G7) and HH inhibitor (e.g., mAb 5E1) administered together with one or more chemotherapeutic agents (see *supra*) to which the tumor type is expected to be responsive, as

described by Ashkenize *et al.*, J. Clin. Invest. 104:155, 1999. The combination of the two agents and chemotherapeutic drug may produce a greater inhibition of tumor growth than either the agents or chemotherapy alone. The effect may be additive or synergistic, and strongly inhibit growth, e.g. by 80% or 90% or more, or even cause tumor regression or disappearance. The HGF and HH inhibitors may also be administered in combination with an antibody against another growth or angiogenic factor, for example anti-VEGF or anti-EGFR, to obtain additive or synergistic growth inhibition and/or tumor regression or disappearance.

2. L2G7 in a medulloblastoma model

[0051] This example utilizes a previously developed model of medulloblastoma in mice (Rao *et al.*, Neoplasia 5:198, 2003). In this model, an avian retroviral vector (RCAS) is used to target gene expression to neural stem cells in the cerebellum of postnatal mice. RCAS is derived from avian leukosis virus (ALV, subgroup A), which normally cannot infect mammalian cells because they lack the cell surface receptor for the virus (TV-A). So a transgenic mouse line (*Ntv-a*) is used, in which the Nestin gene promoter drives expression of the virus receptor. Nestin is an intermediate filament protein expressed by neural stem cells during brain development. In the postnatal cerebellum, the large majority of nestin-expressing neural progenitors are granule neuron precursors (GNPs), which are believed to be the cell population from which medulloblastomas normally arise. Hence, in this transgenic mouse line, primarily GNPs in the cerebellum express the viral receptor for RCAS, so when RCAS is injected into the cerebellum of the newborn mice, it targets and is able to deliver genes to precisely the cells (GNPs) from which medulloblastomas originate.

[0052] It was previously reported that when RCAS was used to deliver the SHH gene in this manner (RCAS-SHH), 3/32 (9%) of the mice developed medulloblastomas and 5/32 showed multifocal hyperproliferation of the external granule layer (EGL) of the cerebellum, a possible precursor stage of medulloblastoma (Rao *et al.*, *op. cit.*). Moreover, when RCAS-SHH and RCAS-Myc carrying the Myc oncogene were injected together, 9/39 (23%) of the mice developed medulloblastomas. In the work described herein, RCAS was used to target expression of HGF, alone and in combination with SHH, to nestin-expressing neural progenitors in the cerebella of newborn mice. After 12 weeks, brain sections were examined for histopathological changes. Injection of RCAS-HGF + RCAS-SHH induced aggressive medulloblastomas in 32/41 mice (78%), a higher incidence than RCAS-SHH alone (16/41 =

39%) ($p = 0.0003$). This suggests that HGF plays a role in medulloblastomas, as it does in gliomas, and in particular HGF expression enhances SHH-dependent medulloblastoma formation.

[0053] In another study, two groups of *Ntv-a* mice were injected with RCAS-HGF + RCAS-SHH on day 0 and then followed for 120 days with treatment by either the L2G7 anti-HGF mAb or a murine isotype-matched control mAb 5G8. As shown in Fig. 2, treatment with L2G7 (i.p., 2.5 mg/kg twice weekly starting on day 14) greatly prolonged survival of the mice relative to the control mAb (median survival > 120 days vs 73 days; $p = 0.03$ by LogRank test applied to the Kaplan-Meier plot). Hence, an HGF inhibitor is effective against medulloblastoma-like tumors that are activated by both HGF and HH.

3. L2G7 and anti-HH mAb in a medulloblastoma model

[0054] A murine mAb that binds and neutralizes at least (human) SHH but preferably both SHH and IHH, and ideally all three HH ligands, is either obtained (e.g., the 5E1 mAb; Ericson et al., *op. cit.*) or developed. It may be developed by art-known methods by immunizing a mouse with all or part of SHH (e.g., as expressed in a baculovirus system), optionally alternating with immunization by IHH, fusing the mouse spleen cells with a fusion partner such as NS myeloma cells, and screening the mAbs from the resulting hybridomas for their ability to bind HH (e.g., by ELISA) and neutralize a biological activity of HH, e.g., the ability of stimulate proliferation of certain cells.

[0055] The anti-HH mAb thus obtained is used to treat *Ntv-a* mice injected with RCAS-HGF + RCAS-SHH as in Example 2 above (with the human SHH gene used in RCAS-SHH), in combination with the L2G7 mAb. In the comparison groups, the mice are treated with only one of anti-HH and L2G7, or neither. The mice are treated with the mAbs twice per week (at typically 5 mg/kg) for a period of time, e.g., from day 7 or 14 through day 28 or 56 or until all the mice in the control group(s) have died, or throughout the course of the experiment. Survival of the mice is monitored, e.g. for 90, 100 or 120 days or longer. Significance of the results is assessed by art-known techniques such as the LogRank test or Cox Proportional Hazard Model applied to a Kaplan-Meier plot. Treatment with L2G7 plus anti-HH mAb provides a statistically significant prolongation of survival relative to treatment with control mAb only, and/or a statistically significant prolongation of survival relative to treatment with only L2G7 or only anti-HH. Optionally, the size of any brain tumors in mice

that have died or been sacrificed may be determined by brain sectioning and immunohistochemistry as has been described (Rao *et al.*, *op. cit.* or Kim *et al.*, *op. cit.*). Treatment with L2G7 plus anti-HH may reduce the size of the brain tumors relative to treatment with neither of these agents or only one.

4. Sequences of preferred anti-HGF mAbs for use in the invention

[0056] As mentioned above, a humanized form of the neutralizing anti-HGF mAb L2G7, e.g., HuL2G7, is especially preferred as the first agent in the invention. The sequences of the heavy and light chains of HuL2G7 are shown in Fig. 3, with the first amino acid of the mature sequences (i.e., the first amino acids of the actual mAb HuL2G7) double underlined. The signal sequences preceding the first amino acid of the heavy and light chains of HuL2G7 are cleaved during expression and secretion. The C-terminal lysine of the heavy chain may be cleaved during expression and processing and may not be present in the final product.

[0057] Also especially preferred for use as the first agent is the anti-HGF mAb 2.12.1 described in WO 2005/017107 A2; the sequences of the variable regions of the light and heavy chains of this mAb are shown in Fig. 4 with the first amino acid of the mature sequences (i.e., the first amino acids of the actual mAb 2.12.1) double underlined. The signal sequences preceding the first amino acid of the heavy and light chains of 2.12.1 are cleaved during expression and secretion. The 2.12.1 mAb has as human constant regions adjoined to these light and heavy chain variable region sequences the human kappa constant region and the human gamma-2 constant region respectively, but mAbs with these variable regions and other human constant regions such as gamma-1 are also preferred for use in the invention. MAbs having light and heavy chain variable regions with the same CDRs as those shown in Fig. 3 or Fig. 4 are also preferred for use in the invention. MAbs that have amino acid sequences 90%, 95% or 99% identical to those shown in Fig. 3 or Fig. 4, at least in the CDRs, when aligned according to the Kabat numbering convention, or which differ from Fig. 3 or Fig. 4 by a small number of functionally inconsequential amino acid substitutions (e.g., conservative substitutions), deletions, or insertions, can also be used in the invention, provided they maintain the functional properties of HuL2G7 or 2.12.1 respectively.

[0058] Although the invention has been described with reference to the presently preferred embodiments, it should be understood that various modifications can be made without

departing from the invention. Unless otherwise apparent from the context any step, element, embodiment, feature or aspect of the invention can be used with any other.

[0059] All publications (including GenBank or UniProtKB/Swiss-Prot Accession numbers and the like), patents and patent applications cited are herein incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent and patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes. In the event of any variance in sequences associated with GenBank and UniProtKB/Swiss-Prot accession numbers and the like, the application refers to the sequences associated with the cited accession numbers as of the priority date of the application (April 11, 2008).

[0060] Applications USSN 61/044,440 and 61/044,446 were filed April 11, 2008 and PCT applications attorney dockets 022382-000510PC and 022382-000710PC filed on the same day as the present application, and are also directed to methods of treating cancer by combination of inhibitors of HGF and a second agent inhibiting a second pathway. Unless otherwise apparent from the context, any step, element, embodiment, feature or aspect of the present application can be combined with any step, element, embodiment, feature or aspect of USSN 61/044,440, 61/044,446, 022382-000510PC, and 022382-000710PC, all of which are incorporated by reference. ATCC Number PTA-5162 has been deposited at the American Type Culture Collection, P.O. Box 1549 Manassas, VA 20108, as ATCC Number PTA-5162 under the Budapest Treaty. This deposit will be maintained at an authorized depository and replaced in the event of mutation, nonviability or destruction for a period of at least five years after the most recent request for release of a sample was received by the depository, for a period of at least thirty years after the date of the deposit, or during the enforceable life of the related patent, whichever period is longest. All restrictions on the availability to the public of these cell lines will be irrevocably removed upon the issuance of a patent from the application.

We claim:

1. A method of treating cancer in a patient by administering to the patient a first agent that is an inhibitor of Hepatocyte Growth Factor (HGF) in combination with a second agent that is an inhibitor of the Hedgehog (HH) cellular signaling pathway.
2. The method of claim 1 wherein said first agent is a monoclonal antibody.
3. The method of claim 2 wherein the monoclonal antibody binds to and neutralizes HGF as a single agent.
4. The method of claim 3 wherein the monoclonal antibody is genetically engineered.
5. The method of claim 4 wherein the monoclonal antibody is human.
6. The method of claim 4 wherein the monoclonal antibody is humanized.
7. The method of claim 6 wherein the monoclonal antibody is a humanized L2G7 antibody.
8. The method of claim 1 wherein the second agent is a monoclonal antibody.
9. The method of claim 8 wherein the monoclonal antibody binds to an HH protein.
10. The method of claim 9 wherein the monoclonal antibody binds to the Sonic Hedgehog protein.
11. The method of claim 10 wherein the monoclonal antibody is genetically engineered.
12. The method of claim 10 wherein the monoclonal antibody is human.
13. The method of claim 10 wherein the monoclonal antibody is humanized.
14. The method of claim 8 wherein the monoclonal antibody binds to the Sonic Hedgehog protein and the Indian Hedgehog protein.

15. The method of claim 14 wherein the monoclonal antibody is genetically engineered.
16. The method of claim 15 wherein the monoclonal antibody is humanized or human.
17. The method of claim 1 wherein the cancer is selected from the group of brain cancer, small cell lung cancer, prostate cancer, breast cancer, and cancers of the digestive tract.
18. The method of claim 1 wherein the cancer is medulloblastoma.
19. The method of claim 4 wherein the cancer is medulloblastoma.
20. The method of claim 11 wherein the cancer is medulloblastoma
21. A composition or kit comprising an inhibitor of hepatocyte growth factor and an inhibitor of the hedgehog signaling pathway.

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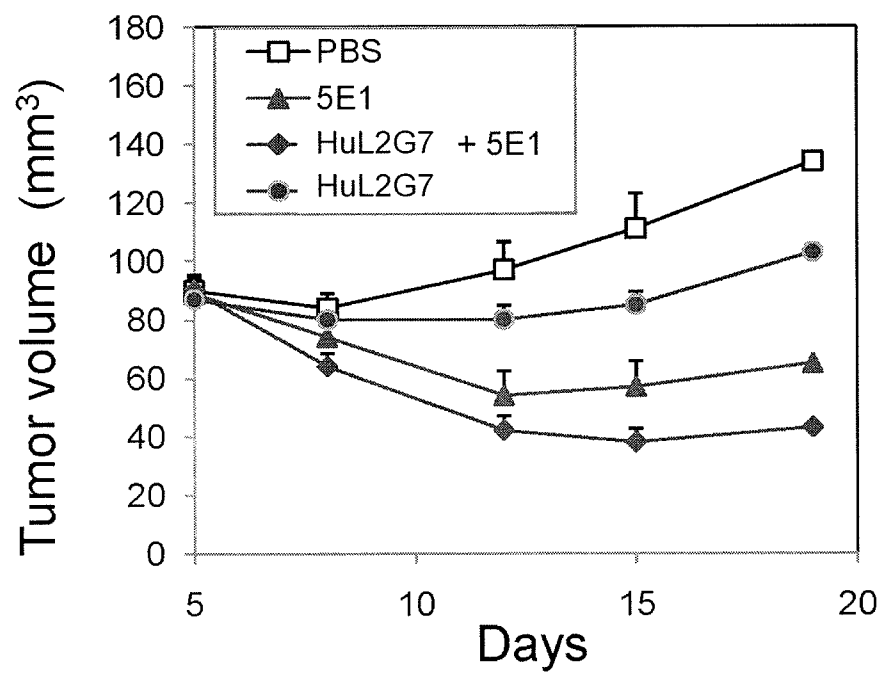


FIGURE 1

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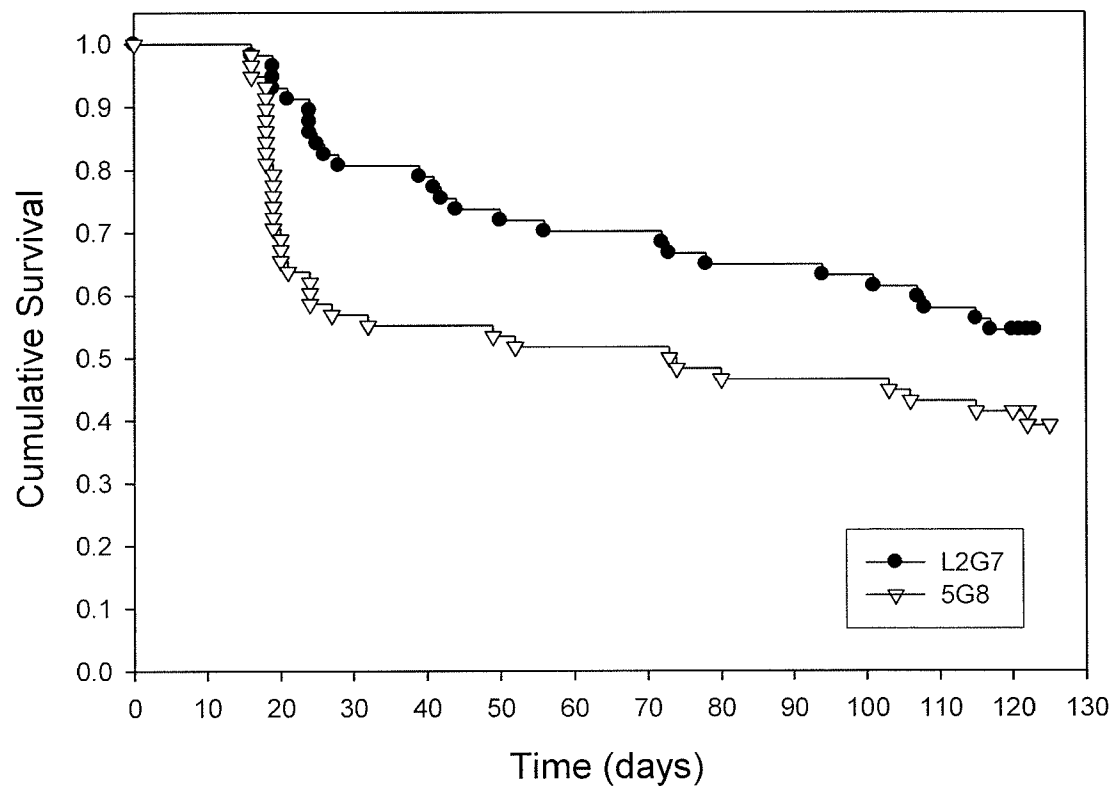


FIGURE 2

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A**1**

-19 M D C T W R I L F L V A A A T G T H A **E** V Q L V Q S G A E V
 12 K K P G A S V K V S C K V S G Y T F S G N W I E W V R Q A P
 42 G K G L E W I G E I L P G S G N T N Y N E K F K G K A T M T
 72 A D T S T D T A Y M E L S S L R S E D T A V Y Y C A R G G H
 102 Y Y G S S W D Y W G Q G T L V T V S S **A** S T K G P S V F P L
 132 A P S S K S T S G G T A A L G C L V K D Y F P E P V T V S W
 162 N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V
 192 P S S S L G T Q T Y I C N V N H K P S N T K V D K R V **E** P K
 222 S C D K T H T C P P C P **A** P E L L G G P S V F L F P P K P K
 252 D T L M I S R T P E V T C V V V D V S H E D P E V K F N W Y
 282 V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V
 312 L H Q D W L N G K E Y K C K V S N K A L P A P I E K T I S K
 342 A K G **Q** P R E P Q V Y T L P P S R E E M T K N Q V S L T C L
 372 V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L
 402 D S D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M
 432 H E A L H N H Y T Q K S L S L S P G K

B**1**

-20 M E A P A Q L L F L L L L W L P D T H G **D** I V M T Q S P S S
 11 L S A S V G D R V T I T C K A S E N V V T Y V S W Y Q Q K P
 41 G K A P K L L I Y G A S N R Y T G V P D R F S G S G S G T D
 71 F T L T I S S L Q P E D F A T Y Y C G Q G Y S Y P Y T F G Q
 101 G T K L E I K R **T** V A A P S V F I F P P S D E Q L K S G T A
 131 S V V C L L N N F Y P R E A K V Q W K V D N A L Q S G N S Q
 161 E S V T E Q D S K D S T Y S L S S T L T L S K A D Y E K H K
 191 V Y A C E V T H Q G L S S P V T K S F N R G E C

FIGURE 3

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A

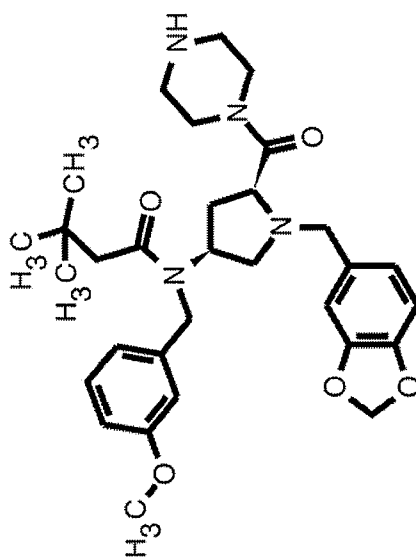
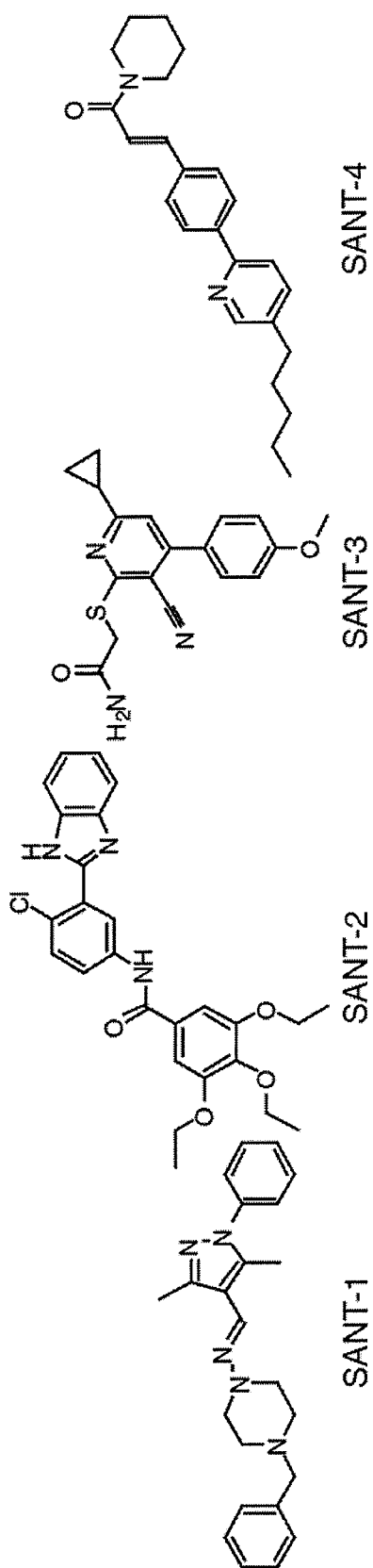
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L S V S P G E R A T L S C R A S Q S V D S N L A W Y R Q K P
G Q A P R L L I Y G A S T R A T G I P A R F S G S G S G T E
F T L T I S S L Q S E D F A V Y Y C Q Q Y I N W P P I T F G
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B

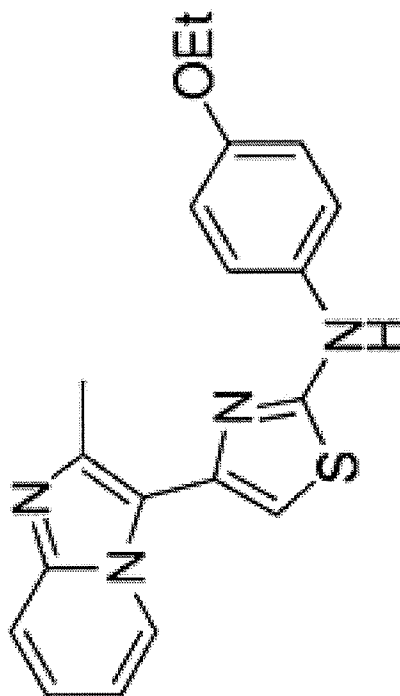
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V K P S E T L S L T C T V S G G S I S I Y Y W S W I R Q P P
G K G L E W I G Y V Y Y S G S T N Y N P S L K S R V T I S V
D T S K N Q F S L K L N S V T A A D T A V Y Y C A R G G Y D
F W S G Y F D Y W G Q G T L V T V S S

FIGURE 4

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CUR61414



JK184

Figure 5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 09/40120

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/00 (2009.01)

USPC - 514/2

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)
USPC - 514/2

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)
WEST -- PGPB,USPT,USOC,EPAB,JPAB; Dialog Classic Files -- 654, 652, 351, 349, 315, 6, 35, 65, 155; Google Scholar; USPTO Web Page; Search terms -- HGF inhibitor, hedgehog signaling protein inhibitor, treating cancer, medulloblastoma, monoclonal antibody, L2G7, human antibody, humanized antibody

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2007/0009530 A1 (ALTABA et al.) 11 January 2007 (11.01.2007) para [0002], [0012], [0016], [0020], [0028], [0035], [0085], [0121], [0126], [0317]	1, 17, 18, 21 ----- 2-16, 19, 20
Y	US 2008/0019974 A1 (KIM et al.) 24 January 2008 (24.01.2008) para [0003], [0012], [0013], [0042], [0050]	2-7, 19
Y	US 2004/0110663 A1 (DUDEK et al.) 10 June 2004 (10.06.2004) para [0027], [0031], [0119], [0121], [0528], [0537], [0540]	8-16, 20

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

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"O" document referring to an oral disclosure, use, exhibition or other means

"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

29 June 2009 (29.06.2009)

Date of mailing of the international search report

10 JUL 2009

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