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(71) Applicant: **CHONG KUN DANG PHARMACEUTICAL CORP.** [KR/KR]; 8, Chungjeong-ro, Seodaemun-gu, Seoul 03742 (KR).

(72) Inventors: **MOON, Seung Kee**; 315-20, Dongbaekjukjeon-daero, Giheung-gu, Yongin-si, Gyeonggi-do 16995 (KR). **LEE, Kyung Woo**; 315-20, Dongbaekjukjeon-daero, Giheung-gu, Yongin-si, Gyeonggi-do 16995 (KR). **JEON, Eun Ju**; 315-20, Dongbaekjukjeon-daero, Giheung-gu, Yongin-si, Gyeonggi-do 16995 (KR). **AN, Ki Young**; 315-20, Dongbaekjukjeon-daero, Giheung-gu, Yongin-si, Gyeonggi-do 16995 (KR). **CHOI, Eun Su**; 315-20, Dongbaekjukjeon-daero, Giheung-gu, Yongin-si, Gyeonggi-do 16995 (KR).

(74) Agent: **AHN, So Young**; 3rd Fl., 416, Nonhyeon-ro, Gangnam-gu, Seoul 06224 (KR).

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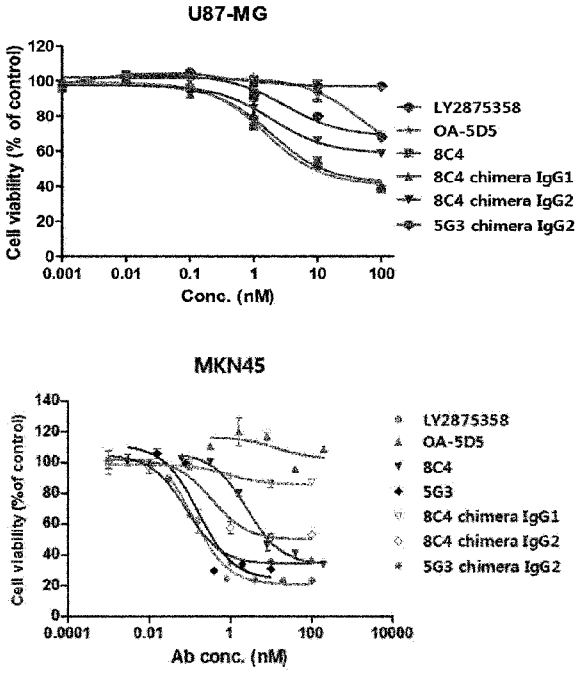

(57) Abstract: The present invention relates to a novel antibody or an antigen binding fragment thereof that specifically binds to a human hepatocyte growth factor receptor (c-Met), and a composition for preventing or treating cancer, wherein the antibody shows an excellent cancer cell proliferation inhibitory activity and a remarkably excellent anticancer activity even by a little amount thereof, thus effectively preventing or treating cancer.

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Description

Title of Invention: A NOVEL ANTI-C-MET ANTIBODY AND USE THEREOF

Technical Field

[1] The present invention relates to an antibody or an antigen binding fragment thereof, specifically binding to a human hepatocyte growth factor receptor (c-Met), and a composition for preventing or treating cancer comprising the same.

[2]

Background Art

[3] Receptor tyrosine kinases (RTK) act as a vital modulator in cell growth, differentiation, neovascularization, tissue recovery, etc. Besides such general physiological processes, an abnormal expression of a certain RTK is associated with the development and progression of many kinds of cancer. Thus, such RTK has been considered as a promising drug target for cancer treatment.

[4] A hepatocyte growth factor receptor (HGFR; c-Met), which is a kind of the RTK, is a receptor on the surface of cells with regard to hepatocyte growth factor known as a scatter factor (HGF/SF) (Laird AD et al., Expert. Opin. Investig. Drugs 12: 51-64 (2003)). An abnormal c-Met activation by HGF, which is one of the representative oncogenic mechanisms, is known to be associated with tumor proliferation, apoptosis inhibition, neovascularization, invasion, metastasis and the like (Bottaro DP et al., Science 251: 802-804 (1991), Day RM et al., Oncogene 18: 3399-3406 (1999)). And also, it is reported that the abnormal c-Met activation by c-Met mutation and amplification is associated with various cancers such as lung cancer, colon cancer, head and neck cancer, stomach cancer, breast cancer, etc., and is also involved in an increase in tumor aggressiveness and its unfavorable prognosis (Lefebvre J et al., FASEB J 26: 1387-1399 (2012), Liu X et al., Trends Mol Med 16: 37-45 (2010), Smolen GA et al., Proc Natl Acad Sci USA 103: 2316-2321 (2006), Foveau B et al., Mol Biol Cell 20: 2495-2507 (2009)).

[5]

[6] Thus, c-Met has drawn much attention as a target antigen for treating such various cancers and various approaches have been made to inhibit the expression and activity of c-Met. As a c-Met-specific small molecule tyrosine kinase inhibitor, which has been known so far, there are Tivantinib (ArQule), INC280 (Novatis), AMG337 (Amgen), etc. And, Rilotumumab (Amgen), Ficlatuzumab (AVEP Pharmaceuticals), HuL2G7 (Galaxy Biotech), etc., have been developed as an HGF-specific monoclonal antibody, which is a ligand of c-Met. Also, as an antagonist monoclonal antibody, which targets

c-Met, there are Onartuzumab (WO 2006/015371) in clinical phase III of development by Genentech, Emibetuzumab (WO 2010/059654) in clinical phase II by Lilly, SAIT-301 (US 2014154251) in clinical phase I of development, ABT-700 (Wang J et al., BMC Cancer. 16: 105-118(2016)), etc. Onartuzumab is a monovalent antagonistic antibody derived from a bivalent monoclonal antibody (5D5), which acts on c-Met as an agent (Mark Merchant, et al., Proc Natl Acad Sci U S A. 110(32): E2987-E299 (2013)). As such, various drugs have been developed with regard to c-Met, but c-Met is associated with the occurrence and progression of various cancers as described above, thus it is constantly driving a continuous demand for developing a new therapeutic agent capable of treating cancer by targeting c-Met.

[7]

Disclosure of Invention

Technical Problem

[8] The present inventors have developed a novel anti-c-Met antibody binding to c-Met with a high affinity and have also identified that such anti-c-Met antibody, a chimera thereof and humanized and affinity-optimized antibodies remarkably inhibit a proliferation of tumor cells and have an excellent anticancer effect, thus having completed the present invention.

[9]

Solution to Problem

[10] One objective of the present invention is to provide an antibody or an antigen binding fragment thereof that specifically binds to a hepatocyte growth factor receptor (c-Met).

[11] Another objective of the present invention is to provide a nucleic acid molecule encoding the antibody or the antigen binding fragment thereof, an expression vector comprising the nucleic acid molecule, a host cell having the expression vector introduced therein, a method for producing an antibody or an antigen binding fragment thereof using the host cell.

[12] Yet another objective of the present invention is to provide a composition for detecting c-Met comprising the antibody or the antigen binding fragment thereof, a kit for detection comprising the same, and a method for detecting a c-Met antigen using the same.

[13] Still yet another objective of the present invention is to provide a composition for preventing or treating cancer comprising the antibody or the antigen binding fragment thereof.

Advantageous Effects of Invention

[14] The antibody or the antigen binding fragment thereof of the present invention that specifically binds to a hepatocyte growth factor receptor (c-Met), has a novel sequence,

and shows an excellent cancer cell proliferation inhibitory activity and a remarkably excellent anticancer activity even by a little amount thereof, thus effectively preventing or treating the disease such as cancer.

[15]

Brief Description of Drawings

[16] FIG. 1 shows results of an in vitro test on tumor cell proliferation inhibitory activity of hybridoma c-Met antibody of the present invention.

[17] FIG. 2 shows a schematic diagram of a vector for expressing a separate transcriptome for scFv display.

[18] FIG. 3 shows results of analyzing a tumor cell proliferation inhibitory activity by hu8C4 affinity-optimized antibody of the present invention.

[19] FIG. 4 shows results of analyzing a tumor cell proliferation inhibitory activity by a bispecific antibody of the present invention.

[20] FIG. 5 shows results of analyzing a tumor cell proliferation inhibitory activity by a bispecific antibody of the present invention.

[21] FIG. 6 shows results of comparing a tumor cell proliferation inhibitory activity between the bispecific antibody of the present invention and a combined therapy in U-87 MG (glioblatoma), NCI-H292 (NSCLC), NCI-H1648 (NSCLC) and NCI-H596 (NSCLC) cell lines.

[22] FIG. 7 shows results of comparing a tumor cell proliferation inhibitory activity between the bispecific antibody of the present invention and a combined therapy in LS174T (colon), BT20 (TNBC) and KP4 (pancreatic) cell lines.

[23] FIG. 8 shows results of comparing a tumor cell proliferation inhibitory activity between the bispecific antibody of the present invention and a combined therapy in HCC827 (NSCLC) and NCI-H596 (NSCLC) cell lines.

[24] FIG. 9 shows results of measuring a binding capacity of the anti-c-Met antibody and the bispecific antibody of the present invention with regard to various kinds of c-Met and EGFR antigens by an ELISA method.

[25] FIG. 10 shows results of measuring an effect of decreasing a receptor level by the bispecific antibody of the present invention in an NCI-H820 (NSCLC) cell line.

[26] FIG. 11 shows results of measuring an inhibition of c-Met and EGFR phosphorylation by the anti-c-Met antibody and the bispecific antibody of the present invention in an NCI-H820 (NSCLC) cell line.

[27] FIG. 12 shows results of measuring an anticancer effect of the bispecific antibody of the present invention in a U-87 MG (glioblastoma) cell xenograft model.

[28] FIG. 13 shows results of measuring an anticancer effect of the bispecific antibody of the present invention in an NCI-H820 (NSCLC) cell xenograft model.

[29] FIG. 14 shows results of analyzing a tumor cell proliferation inhibitory activity by treating the anti-c-Met antibody of the present invention and the anti-HER2 antibody by a combined therapy in an NCI-H2170 (NSCLC) cell line.

[30] FIG. 15 shows results of measuring an anticancer effect of a combined therapy with the anti-c-Met antibody of the present invention and the anti-HER2 antibody in an NCI-H2170 (NSCLC) cell xenograft model.

[31] FIG. 16 shows results of measuring an anticancer effect of the bispecific antibody of the present invention in an NCI-H596 (NSCLC) cell xenograft model.

[32] FIG. 17 shows results of measuring an anticancer effect of the bispecific antibody of the present invention in an EBC-1 (NSCLC) cell xenograft model.

[33] FIG. 18 shows results of indicating an amount of c-Met on the surface of cells, measured after treating an HCC827 cell line with a bispecific antibody (hu8C4 x Vectibix scFv), etc.

[34] FIG. 19 shows results of indicating an amount of EGFR on the surface of cells, measured after treating an HCC827 cell line with a bispecific antibody (hu8C4 x Vectibix scFv), etc.

[35] FIG. 20 shows results of indicating an epitope of a bispecific antibody, analyzed by a hydrogen-deuterium exchange mass spectrometry (HDX-MS), in a tertiary structure.

[36]

Best Mode for Carrying out the Invention

[37] Hereinafter, the present invention will be described in more detail as follows. Meanwhile, each description and embodiment disclosed in the present invention may be applied to other descriptions and embodiments respectively as well. In other words, all the combinations of various elements disclosed in the present invention are within the scope of the present invention. Also, the scope of the present invention may not be restricted by the detailed descriptions below.

[38]

[39] To achieve the objectives above, one aspect of the present invention provides an antibody or an antigen binding fragment thereof that specifically binds to a hepatocyte growth factor receptor (c-Met).

[40] The antibody or the antigen binding fragment thereof of the present invention, specifically binding to c-Met, binds to c-Met with a high affinity to inhibit an expression or activity thereof, thus showing an excellent tumor cell proliferation inhibitory activity, such that the antibody alone or with conventional pharmaceutically acceptable carriers, other anticancer drugs, anticancer adjuvants, etc. may be valuably used as an anticancer composition for preventing or treating cancer.

[41] In the present invention, the term "antibody" means a protein molecule serving as a

receptor for specifically recognizing an antigen, comprising an immunoglobulin molecule immunologically having reactivity with a certain antigen, wherein examples thereof may comprise a monoclonal antibody, a polyclonal antibody, a full-length antibody and antibody fragments all. Also, the term may comprise a bivalent or bispecific molecule (e.g., a bispecific antibody), a diabody, a triabody or a tetrabody.

[42] In the present invention, the term "monoclonal antibody" refers to an antibody molecule of a single molecule composition obtained from substantially the same antibody population, wherein such monoclonal antibody shows a single binding specificity and affinity for a certain epitope. In the present invention, the term "full-length antibody" has a structure with two full-length light chains and two full-length heavy chains, wherein each of light chains is linked to a heavy chain by a disulfide bond. A constant region of the heavy chain has gamma (γ), mu (μ), alpha (α), delta (δ) and epsilon (ε) types, and also has gamma1 (γ1), gamma2 (γ2), gamma3 (γ3), gamma4 (γ4), alpha1 (α1) and alpha2 (α2) as a subclass. A constant region of the light chain has kappa (κ) and lambda (λ) types. IgG comprises IgG1, IgG2, IgG3 and IgG4 as a subtype.

[43] In the present invention, the terms "fragment," "antibody fragment" and "antigen binding fragment" refer to any fragments of the antibody of the present invention having an antigen binding function of the antibody, wherein such terms are used inter-changeably with each other. Exemplary antigen binding fragments comprise Fab, Fab', $F(ab')_2$, Fv and the like, but not limited thereto.

[44] The Fab has a structure with a variable region of light and heavy chains, a constant region of light chain and a first constant region of heavy chain (CH1 domain), and also has one antigen binding site. An antigen binding fragment of an antibody molecule or an antibody fragment means a fragment having an antigen binding function, and Fab' is different from Fab in that the former has a hinge region having one or more cysteine residue in C terminus of a heavy chain CH1 domain. $F(ab')_2$ antibody is created in such a way that a cysteine residue of a hinge region of Fab' forms a disulfide bond. Fv is a minimal antibody fragment having only a heavy chain variable region and a light chain variable region, wherein a recombinant technology for creating Fv fragments is disclosed in PCT International Patent Publication Applications WO 88/10649, WO 88/106630, WO 88/07085, WO 88/07086, WO 88/09344 and the like. Two-chain Fv is formed in such a way that a heavy chain variable region and a light chain variable region are linked to each other by a non-covalent bond, while single-chain Fv is formed in such a way that a heavy chain variable region and a single chain variable region are generally linked with each other either by a covalent bond through a peptide linker or directly linked in C-terminus, thus forming a structure like a dimer as shown in the two-chain Fv. Such antibody fragment may be obtained by using a protein

hydrolase (for example, Fab may be obtained by performing a restriction digestion of a whole antibody by papain and $F(ab')_2$ fragment may be obtained by performing a digestion of the same by pepsin) or may be produced by a gene recombination technology, but not limited thereto.

[45]

[46] Particularly in the present invention, it may be provided that the antibody specifically binding to c-Met is:

[47] (a) an antibody comprising a light chain variable region comprising a light chain CDR1 represented by SEQ ID NO: 1; a light chain CDR2 represented by SEQ ID NO: 2; a light chain CDR3 represented by SEQ ID NO: 3, and a heavy chain variable region comprising a heavy chain CDR1 represented by SEQ ID NO: 7; a heavy chain CDR2 represented by SEQ ID NO: 8; and a heavy chain CDR3 represented by SEQ ID NO: 9;

[48] (b) an antibody comprising a light chain variable region comprising a light chain CDR1 represented by SEQ ID NO: 4; a light chain CDR2 represented by SEQ ID NO: 5; a light chain CDR3 represented by SEQ ID NO: 6, and a heavy chain variable region comprising a heavy chain CDR1 represented by SEQ ID NO: 10; a heavy chain CDR2 represented by SEQ ID NO: 11; and a heavy chain CDR3 represented by SEQ ID NO: 12; or

[49] (c) affinity-optimized antibodies thereof.

[50] In the present invention, the term "heavy chain" may comprise both a full-length heavy chain and a fragment thereof comprising a variable region domain VH with an amino acid sequence having a variable region sequence enough to give specificity to an antigen, as well as three constant region domains CH1, CH2 and CH3. Also, in the present invention, the term "light chain" may comprise both a full-length light chain and a fragment thereof comprising a variable region domain VL with an amino acid sequence having a variable region sequence enough to give specificity to an antigen, as well as a constant region domain CL.

[51] In the present invention, the antibody may comprise both a mouse antibody produced from a mouse, and a mutant thereof, wherein a part of an amino acid sequence of a parent antibody is substituted, added and/or deleted to improve the affinity, immunity, etc., of the antibody. The mutant may comprise a chimeric antibody, a humanized antibody, an affinity-optimized antibody, etc., as an example, but not limited thereto. In the present invention, the mutant comprehensively refers to an antibody, wherein a part of a CDR amino acid sequence of a parent antibody is mutated (substituted, added or deleted) on condition of having the same CDR as that of the parent antibody or targeting the same epitope as that of the parent antibody. Such mutant may be appropriately adjusted by those skilled in the art to improve the affinity, immunity and the

like of an antibody within the scope of maintaining a binding capacity for the same epitope.

[52] In other words, the antibody or the antigen binding fragment thereof of the present invention may comprise a sequence of anti-c-Met antibody described herein as well as biological equivalents thereof, within the scope of specifically recognizing c-Met. For example, an additional change may be made in an amino acid sequence of the antibody, in order to further improve the binding affinity and/or other biological characteristics of the antibody. Such change comprises, for example, the deletion, insertion and/or substitution of an amino acid sequence residue of the antibody. Such amino acid mutation is made based on relative similarity of amino acid side chain substituent, e.g., hydrophobicity, hydrophilicity, charge, size, etc. By analyzing the size, shape and type of amino acid side chain substituent, it can be seen that arginine, lysine and histidine are all positive charge residues; alanine, glycine and serine have a similar size; and phenylalanine, tryptophan and tyrosine have a similar shape. Thus, based on such considerations, it can be seen that arginine, lysine and histidine; alanine, glycine and serine; and phenylalanine, tryptophan and tyrosine are biologically functional equivalents.

[53] In the present invention, the term "chimeric antibody" is an antibody formed in such a way that a variable region of a mouse antibody is recombined with a constant region of a human antibody, which results in a greatly improved immune reaction in comparison with a mouse antibody.

[54] In the present invention, the term "humanized antibody" means an antibody formed in such a way that a protein sequence of an antibody derived from other species than human is modified to be similar to that of an antibody mutant naturally produced from human. For example, the humanized antibody may be prepared by preparing a humanized variable region through a recombination of CDR derived from a mouse with FR derived from a human antibody and then by recombining the same with a constant region of a preferred human antibody. However, a simple CDR grafting only results in a low affinity of the humanized antibody, so several key FR amino acid residues, which are considered to possibly influence a three-dimensional structure of CDR, may develop an affinity with those of mouse antibody, thus reaching the same level as the affinity of an original mouse antibody.

[55] In the present invention, the term "affinity-optimized antibody," which is a mutant formed in such a way that a part of CDR sequence of a certain antibody is substituted, added or deleted, means an antibody with a better binding affinity to an antigen while binding to the same antigen epitope as that of the certain antibody. Particularly, the affinity-optimized antibody of the present invention refers to a mutant antibody binds to the same epitope as that of: (a) an antibody comprising a light chain variable region

comprising a light chain CDR1 represented by SEQ ID NO: 1; a light chain CDR2 represented by SEQ ID NO: 2; a light chain CDR3 represented by SEQ ID NO: 3, and a heavy chain variable region comprising a heavy chain CDR1 represented by SEQ ID NO: 7; a heavy chain CDR2 represented by SEQ ID NO: 8; a heavy chain CDR3 represented by SEQ ID NO: 9; or (b) an antibody comprising a light chain variable region comprising a light chain CDR1 represented by SEQ ID NO: 4; a light chain CDR2 represented by SEQ ID NO: 5; a light chain CDR3 represented by SEQ ID NO: 6, and a heavy chain variable region comprising a heavy chain CDR1 represented by SEQ ID NO: 10; a heavy chain CDR2 represented by SEQ ID NO: 11; a heavy chain CDR3 represented by SEQ ID NO: 12. A person of ordinary skill in the art may prepare the affinity-optimized antibody by using a known technology based on certain light chain and heavy chain CDR sequences. For example, the affinity-optimized antibody of the present invention may be prepared through a phage display. In the present invention, the term "phage display" refers to a technology, which displays a mutant polypeptide as a fusion protein with at least a part of coat protein on a phage, for example, on the surface of fibrous phage particles. The usefulness of the phage display lies in the fact that it targets a large library of randomized protein mutants, thus promptly and efficiently classifying sequences binding to a target antigen with a high affinity. Displaying a library of peptides and proteins on the phage has been used for screening millions of polypeptides in order to see a polypeptide with a specific binding characteristic.

[56]

[57] In one exemplary embodiment of the present invention, it may be provided that the antibody is an antibody comprising: (a) a light chain variable region represented by SEQ ID NO: 13 and a heavy chain variable region represented by SEQ ID NO: 15; or (b) a light chain variable region represented by SEQ ID NO: 14 and a heavy chain variable region represented by SEQ ID NO: 16. As an example, it may be provided that the antibody is an antibody comprising: (a) a light chain variable region coded by a nucleotide represented by SEQ ID NO: 17 and a heavy chain variable region coded by a nucleotide represented by SEQ ID NO: 19; or (b) a light chain variable region coded by a nucleotide represented by SEQ ID NO: 18 and a heavy chain variable region coded by a nucleotide represented by SEQ ID NO: 20, but not limited thereto.

[58] According to one specific embodiment of the present invention, a hybridoma cell group was obtained from a mouse, wherein a human c-Met Sema domain/Fc fusion protein is an antigen, from which anti-c-Met antibody specifically binding to c-Met was selected by screening with an ELISA analysis method using c-Met/His fusion protein as an antigen. The selected antibody and the chimeric antibody thereof have a tumor cell proliferation inhibitory activity, which is equal to or more excellent than

even commercially available known LY2875358 and OA-5D5 (Table 3 and FIG. 1), thus being very valuably used in prevention or treatment of cancer.

[59]

[60] In another exemplary embodiment of the present invention, it may be provided that the antibody comprises:

[61] (a) a light chain variable region represented by SEQ ID NO: 21 and a heavy chain variable region represented by SEQ ID NO: 23; (b) a light chain variable region represented by SEQ ID NO: 22 and a heavy chain variable region represented by SEQ ID NO: 24; (c) a light chain variable region represented by SEQ ID NO: 29 and a heavy chain variable region represented by SEQ ID NO: 31; or (d) a light chain variable region represented by SEQ ID NO: 30 and a heavy chain variable region represented by SEQ ID NO: 32. As an example, it may be provided that the antibody is an antibody comprising: (a) a light chain variable region coded by a nucleotide represented by SEQ ID NO: 25 and a heavy chain variable region coded by a nucleotide represented by SEQ ID NO: 27; (b) a light chain variable region coded by a nucleotide represented by SEQ ID NO: 26 and a heavy chain variable region coded by a nucleotide represented by SEQ ID NO: 28; (c) a light chain variable region coded by a nucleotide represented by SEQ ID NO: 33 and a heavy chain variable region coded by a nucleotide represented by SEQ ID NO: 35; or (d) a light chain variable region coded by a nucleotide represented by SEQ ID NO: 34 and a heavy chain variable region coded by a nucleotide represented by SEQ ID NO: 36, but not limited thereto. Also, it may be provided that the antibody comprises a hinge region represented by one of SEQ ID NO: 37 to SEQ ID NO: 44.

[62] In one specific embodiment of the present invention, a humanized antibody comprising CDR of the antibody obtained through a phage display selection was prepared, and it was identified that such antibody showed an anticancer activity, which was similar to that of the chimera antibody of the present invention (Examples 2 and 3). Also, in another specific embodiment of the present invention, a tumor cell proliferation inhibitory activity of the antibody was evaluated according to a hinge region sequence, and it was identified that a proliferation of most tumor cells was effectively inhibited, even with a somewhat difference in the activity depending on the difference of hinge sequence (Table 7).

[63]

[64] In yet another exemplary embodiment of the present invention, but not limited thereto, it may be provided that an affinity-optimized antibody for the humanized antibody is an antibody, wherein one or more amino acid sequence is substituted from an antibody comprising: a light chain variable region comprising a light chain CDR1 represented by SEQ ID NO: 1; a light chain CDR2 represented by SEQ ID NO: 2; a

light chain CDR3 represented by SEQ ID NO: 3, and a heavy chain variable region comprising a heavy chain CDR1 represented by SEQ ID NO: 7; a heavy chain CDR2 represented by SEQ ID NO: 8; a heavy chain CDR3 represented by SEQ ID NO: 9, and wherein, (i) G in a 1st position of the light chain CDR1 is substituted with A, E, K, L, N, R, S, V or W; A in a 2nd position thereof is substituted with C, G, I, P, S, T or V; S in a 3rd position thereof is substituted with G, M, N, P, Q, R, S or T; E in a 4th position thereof is substituted with A, D, F, G, H, K, M, Q, R, S, T or V; N in a 5th position thereof is substituted with A, D, E, G, K, L, P, Q, R, S, T or V; I in a 6th position thereof is substituted with A, F, L, M, Q, R, S, T or V; Y in a 7th position thereof is substituted with F, H, R or V; or G in a 8th position thereof is substituted with D, F, H, M, N, R, S, T or V; (ii) G in a 1st position of the light chain CDR2 is substituted with D, F, H, K, P, Q, S, V or Y; T in a 3rd position thereof is substituted with Q; or N in a 4th position thereof is substituted with G; (iii) Q in a 1st position of the light chain CDR3 is substituted with E, G, I, M or N; N in a 2nd position thereof is substituted with A, D, E, H, L, Q, S or T; V in a 3rd position thereof is substituted with I, L, M, N, Q, S or T; L in a 4th position thereof is substituted with F, H, I, M, R, S, V, W or Y; S in a 5th position thereof is substituted with C, D, E, F, G, H, K, L, N, Q, R, T, V or Y; S in a 6th position thereof is substituted with D, E, F, G, H, I, L, M, N, P, Q, R, T, V or Y; P in a 7th position thereof is substituted with A, D, E, G, N, Q, S or V; Y in an 8th position thereof is substituted with E, F, L, M or Q; or T in a 9th position thereof is substituted with D, F, G, I, L, N, S, V, W or Y; (iv) D in a 1st position of the heavy chain CDR1 is substituted with G or Q; Y in a 2nd position thereof is substituted with Q; or I in a 4th position thereof is substituted with A or Q; (v) F in a 3rd position of the heavy chain CDR2 is substituted with D, E, W or Y; G in a 5th position thereof is substituted with D, H or Y; S in a 6th position thereof is substituted with F, P, W or Y; G in a 7th position thereof is substituted with A, F, L, N or T; N in an 8th position thereof is substituted with F, P, S, T or Y; T in a 9th position thereof is substituted with A, D, E, F, G, H, L, P, S or V; H in a 10th position thereof is substituted with A, D, F, M, R, S, T, V, W or Y; F in an 11th position thereof is substituted with G, H, I, L, M, N, P, Q, V or Y; S in a 12th position thereof is substituted with A, D, G, H, I, L, P, T or V; A in a 13th position thereof is substituted with D, E, F, G, H, I, K, L, M, P, R, S, T, V or Y; R in a 14th position thereof is substituted with A, E, G, H, L, N, P, Q, S, W or Y; F in a 15th position thereof is substituted with D, E, G, L, M, P, R, S, V or W; K in a 16th position thereof is substituted with A, E, F, G, H, L, R, S, T, V or Y; or G in a 17th position thereof is substituted with E, F, H, L, M, N, P, Q, R, S, T, V or W; or (vi) G in a 1st position of the heavy chain CDR3 is substituted with E, F, H, N, Q, V or W; D in a 2nd position thereof is substituted with E; Y in a 3rd position thereof is substituted with L, Q, T or V; G in a 4th position thereof is substituted with W; F in a 5th

position thereof is substituted with L or Y; L in a 6th position thereof is substituted with Q, S or Y; or Y in a 7th position thereof is substituted with C, L, M, N or Q. Herein, it may be provided that the light chain CDR1 comprises 0 to 5 substitutions, the light chain CDR2 comprises 0 to 1 substitution, the light chain CDR3 comprises 0 to 7 substitutions, the heavy chain CDR1 comprises 0 to 1 substitution, the heavy chain CDR2 comprises 0 to 11 substitutions, and the heavy chain CDR3 comprises 0 to 6 substitutions.

[65] Particularly, in still yet another exemplary embodiment of the present invention, it may be provided that the affinity-optimized antibody comprises a light chain variable region comprising a light chain CDR1 represented by any one of SEQ ID NO: 1 and SEQ ID NO: 229 to SEQ ID NO: 268; a light chain CDR2 represented by any one of SEQ ID NO: 2, SEQ ID NO: 182 to SEQ ID NO: 190, SEQ ID NO: 227 and SEQ ID NO: 228; a light chain CDR3 represented by any one of SEQ ID NO: 3, SEQ ID NO: 142 to SEQ ID NO: 181, SEQ ID NO: 191 to SEQ ID NO: 226 and SEQ ID NO: 269 to SEQ ID NO: 301; and a heavy chain variable region comprising a heavy chain CDR1 represented by any one of SEQ ID NO: 7 and SEQ ID NO: 108 to SEQ ID NO: 112; a heavy chain CDR2 represented by any one of SEQ ID NO: 8, SEQ ID NO: 54 to SEQ ID NO: 63, SEQ ID NO: 72 to SEQ ID NO: 107 and SEQ ID NO: 118 to SEQ ID NO: 141; a heavy chain CDR3 represented by any one of SEQ ID NO: 9, SEQ ID NO: 64 to SEQ ID NO: 71 and SEQ ID NO: 113 to SEQ ID NO: 117, more particularly, comprising a light chain variable region represented by any one of SEQ ID NO: 21 and SEQ ID NO: 306 to SEQ ID NO: 311, and a heavy chain variable region represented by any one of SEQ ID NO: 23 and SEQ ID NO: 302 to SEQ ID NO: 305, and much more particularly comprising: (a) a light chain variable region represented by SEQ ID NO: 21 and a heavy chain variable region represented by SEQ ID NO: 302; (b) a light chain variable region represented by SEQ ID NO: 21 and a heavy chain variable region represented by SEQ ID NO: 305; (c) a light chain variable region represented by SEQ ID NO: 310 and a heavy chain variable region represented by SEQ ID NO: 23; (d) a light chain variable region represented by SEQ ID NO: 308 and a heavy chain variable region represented by SEQ ID NO: 305; (e) a light chain variable region represented by SEQ ID NO: 306 and a heavy chain variable region represented by SEQ ID NO: 303; (f) a light chain variable region represented by SEQ ID NO: 307 and a heavy chain variable region represented by SEQ ID NO: 304; (g) a light chain variable region represented by SEQ ID NO: 308 and a heavy chain variable region represented by SEQ ID NO: 304; (h) a light chain variable region represented by SEQ ID NO: 309 and a heavy chain variable region represented by SEQ ID NO: 304; (i) a light chain variable region represented by SEQ ID NO: 311 and a heavy chain variable region represented by SEQ ID NO: 304; or (j) a light chain variable region represented

by SEQ ID NO: 306 and a heavy chain variable region represented by SEQ ID NO: 302, but not limited thereto.

[66] In one specific embodiment of the present invention, a competitive selection method was used to select an antibody with a more improved affinity than the humanized antibody, thus obtaining a number of affinity-optimized antibodies (Tables 8 to 10 and 12). The affinity-optimized antibody has a tumor cell proliferation inhibitory effect that is 4.3 to 28.5 times more excellent than the humanized body (Table 11, 13 and FIG. 3).

[67]

[68] In the present invention, it may be provided that the antibody is an antibody or an antigen binding fragment thereof specifically further binding to an epidermal growth factor receptor (EGFR) in addition to specifically binding to c-Met.

[69] It is known that the EGFR, one of ErbB tyrosine kinases, is abnormally activated in many epidermal cell tumors comprising non-small-cell lung carcinoma, causes cell proliferation, invasion, metastasis and angiogenesis, and increases cell survival. Gefitinib (Iressa), elotinib (Tarceva) and osimertinib (Tagrisso), which are EGFR tyrosine kinase inhibitors, are used as a representative lung cancer therapeutic agent; and cetuximab (Erbitux) and panitumumab (Vectibix), which are EGFR target antibodies, are used as a colon cancer therapeutic agent (Yewale C et al., Biomaterials. 2013 34(34):8690-707 (2013), Deric L. Wheeler et al., Nature Reviews Clinical Oncology 7, 493-507 (2010)).

[70] Such EGFR target therapeutic agents cause resistance one year before and after treatment, wherein c-Met amplification, mutation and HGF-induced activation are known as a key mechanism of resistance (Simona Corso Cancer Discovery 3:978-992 (2013), Curtis R Chong et al., Nature Medicine 19, 1389-1400 (2013)). Also, it is reported that EGFR and c-Met are simultaneously expressed in various tumor cells, wherein, upon inhibiting EGFR, c-Met becomes activated, thus promptly developing the resistance of EGFR TKI (Engelman, J.A., et al., Science, 316:1039-43 (2007)).

[71] Based on such mechanism, a single treatment with a c-Met target drug alone and a combined treatment with an EGFR target drug have been now in a clinical trial, but their efficacy has not been verified yet as a therapeutic agent and there is a need for developing a therapeutic agent for c-Met-related cancerous tumors, known as a key cause of resistance. Accordingly, the present inventors have prepared c-Met/EGFR bispecific antibody based on the antibody described above. The bispecific antibody not only effectively inhibits a proliferation of tumor cells, which are resistant to existing EGFR therapeutic agents, but also shows an excellent proliferation inhibitory activity against tumor cells, thus being valuably used in treatment of diseases such as c-Met-mediated cancers through various mechanisms.

[72] It may be provided that the bispecific antibody is formed in such a way that an

antibody or an antigen binding fragment thereof specifically binding to EGFR is linked to one light chain or heavy chain terminus of c-Met specific antibody, for example, being linked to a heavy chain C-terminus, but not limited thereto.

[73] It may be provided that the binding fragment specifically binding to EGFR is Fab, Fab', $F(ab')_2$ or Fv.

[74] In one exemplary embodiment of the present invention, it may be provided that the Fv is a scFv fragment, wherein the scFv fragment is linked by a connector capable of linking the scFv fragment to one light chain or heavy chain terminus of c-Met antibody. In one exemplary embodiment of the present invention, an antibody specifically binding to EGFR is further prepared by linking with a connector represented by SEQ ID NO: 312.

[75] It may be provided that the EGFR scFv fragment is an EGFR scFv capable of specifically binding to EGFR, known in the art, wherein, for example, there are Erbitux, Vectibix, Portrazza, TheraCIM or the like, but not limited thereto.

[76] In one exemplary embodiment of the present invention, it may be provided that the EGFR scFv is an Erbitux or Vectibix scFv fragment, particularly the EGFR scFv comprises an amino acid sequence represented by SEQ ID NO: 313 or SEQ ID NO: 314, wherein the Vectibix scFv comprises an amino acid sequence represented by SEQ ID NO: 315, but not limited thereto.

[77] According to one specific embodiment of the present invention, as a result of identifying a tumor cell proliferation inhibitory activity of the bispecific antibody, it was identified that the antibody had a more excellent tumor activity inhibitory efficacy than a hu8C4 optimized antibody (Tables 16 and 17, and FIGS. 4, 5, 16 and 17). In particular, it was identified that the antibody of the present invention had an excellent cell proliferation inhibitory effect on even NCI-H292 and NCI-H1648 cell lines, in which c-Met and EGFR are normally expressed (Tables 17 and 19 and FIG. 6). Based on such results, it can be seen that an anticancer effect of the antibody of the present invention is not particularly limited by an abnormality of c-Met expression or a presence or absence of c-Met mutation, etc.

[78] Furthermore, it was identified that the bispecific antibody of the present invention had a more excellent tumor cell proliferation inhibitory capacity than a combined therapy of two antibodies (Tables 18 to 21 and FIGS. 6 to 8). Also, as a result of identifying an effect of the bispecific antibody of the present invention on the activity of antigens and signal transduction materials, it was identified that the bispecific antibody of the present invention had a more excellent signal transduction inhibitory efficacy than an antibody alone (FIG. 11).

[79] It may be provided that the antibody or the antigen binding fragment thereof of the present invention binds to an epitope region represented by an amino acid sequence

selected from the group represented by SEQ ID NO: 331, SEQ ID NO: 332, SEQ ID NO: 333 and/or SEQ ID NO: 334. An affinity-optimized antibody prepared based on a certain antibody (reference antibody) is characterized by having a high homology with the light chain and heavy chain CDR sequences of a variable region with regard to the reference antibody, thus binding to the same epitope region as the reference antibody, such that such affinity-optimized antibody can share all the biological characteristics such as a pharmaceutical mechanism and a pharmaceutical efficacy caused by a binding site, specificity and antibody and exhibit a more excellent effect on binding affinity than the reference antibody.

[80] The epitope region respectively means, for example, YVSKPGAQL (SEQ ID NO: 331) in 321th to 329th positions, IGASLNDDI (SEQ ID NO: 332) in 333th to 341th positions, PIKYVND (SEQ ID NO: 333) in 366th to 372th positions, and QVVVSRSGPST (SEQ ID NO: 334) in 464th to 474th positions from N-terminus of a reference c-Met antigen (SEQ ID NO: 335), wherein c-Met antigen sequence with the antibody or the antigen binding fragment thereof of the present invention binding thereto comprises a partial mutation (substitution, addition or deletion) or a binding antigen exists in a form of a c-Met fragment, precursor or subtype, thus its binding sites or sequences may somewhat vary accordingly. Nevertheless, a person of ordinary skill in the art may clearly specify a position and a sequence, to which the antigen or the antigen binding fragment thereof of the present invention binds based on an epitope sequence information of a reference c-Met antigen.

[81] In one specific embodiment of the present invention, it was identified that the bispecific antibody hu8C4 x Vectibix scFv of the present invention binds to 4 epitope regions of Y321 - L329 (SEQ ID NO: 331), I333 - I341 (SEQ ID NO: 332), P366 - D372 (SEQ ID NO: 333), and Q464 - S474 (SEQ ID NO: 334) of a human c-Met sema domain β chain (Table 28).

[82] The "antibody or antigen binding fragment thereof specifically binding to c-Met" of the present invention means the one binding to a human c-Met by K_D 1 X 10^{-7} M or less. It may be provided that the antibody or the antigen binding fragment thereof binds to human c-Met, for example, by K_D 5 X 10^{-8} M or less, K_D 1 X 10^{-8} M or less, K_D 5 X 10^{-9} M or less, or K_D 1 X 10^{-9} M or less, but not limited thereto.

[83] In one specific embodiment of the present invention, it was directly identified that the antibody or the antigen binding fragments thereof of the present invention had a high binding affinity to c-Met antigen by identifying a binding affinity of hu8C4, hu8C4 AH71 and hu8C4 x Vectibix scFv to c-Met ECD, thus identifying K_D values of 3.173 X 10^{-10}, 9.993 X 10^{-11} and 2.78 X 10^{-10}, respectively (Table 22). It was identified that the antibody or the antigen binding fragment thereof of the present invention had a cross-reactivity to a c-Met antigen of a cynomolgus monkey, which is an ape (Table

22), but did not bind to other animal-derived antigens (e.g., rodents) (FIG. 9). Also, it was identified that the antibody or the antigen binding fragment thereof of the present invention did not bind to other receptors on the surface of cells than c-Met (Table 24). Thus, it can be seen from the results above that the antibody or the antigen binding fragment thereof of the present invention showed a binding specificity to c-Met antigen of humans and monkeys.

[84] As used herein, the term "binding constant (K_{on})" means a binding ratio of a certain antibody-antigen interaction, and the term "dissociation constant (K_{off})" means a dissociation ratio of a certain antibody-antigen interaction. Also, in the present invention, the term "affinity to antigen (K_D)" is the one that a ratio of K_{off} : K_{on} (i.e., K_{off} / K_{on}) is indicated as a molar concentration (M). It may be provided that a K_D value for an antibody is measured by using a method widely established in the art. For example, as a method for measuring a K_D value of an antibody, it may be provided by a surface plasmon resonance analysis using a Biocore™ system, but not limited thereto.

[85] Another aspect of the present invention provides a method for producing a nucleic acid molecule for coding the antibody or the antigen binding fragment thereof, an expression vector comprising the nucleic acid molecule, a host cell having the expression vector introduced therein, an antibody using the host cell or an antigen binding fragment thereof.

[86] The antibody and the antigen binding fragment thereof are such as that described above.

[87] As used herein, the term "nucleic acid molecule" has a meaning that comprehensively comprises DNA and RNA molecules, wherein a nucleotide, a basic constituent unit in the nucleic acid molecule, comprises not only a natural nucleotide, but also an analogue, in which a sugar or base portion is modified (Scheit, Nucleotide Analogs, John Wiley, New York (1980); Uhlman and Peyman, Chemical Reviews, (1990) 90:543-584). A sequence of a nucleic acid molecule for coding the heavy chain and light chain variable regions of the present invention may be modified, wherein the modification comprises an addition, deletion, or non-conservative or conservative substitution of nucleotide.

[88] It is understood that the nucleic acid molecule of the present invention also comprises a nucleotide sequence representing a substantial identity with the aforementioned nucleotide sequence. In the present invention, in case of aligning the aforementioned nucleotide sequence of the present invention with any other sequences in the most corresponding way and analyzing the aligned sequences by an algorithm conventionally used in the art, the substantial identity means a nucleotide sequence that represents a minimal 80% homology, particularly a minimal 90% homology, more particularly a minimal 95% homology.

[89] As used herein, the term "vector," which is a means for expressing a target gene in a host cell, comprises a plasmid vector; a cosmid vector; and virus vector such as a bacteriophage vector, an adenovirus vector, a retrovirus vector and an adeno-related virus, particularly a plasmid vector, but not limited thereto.

[90] In the vector of the present invention, it may be provided that a nucleic acid molecule for coding a light chain variable region and a nucleic acid molecule for coding a heavy chain variable region are operatively linked with a promoter.

[91] In the present invention, the term "operatively linked" means a functional binding between a nucleic acid expression regulatory sequence (e.g., a promoter, a signal sequence, or an array in a transcriptional regulatory factor binding site) and other nucleic acid sequence, thus the regulatory sequence controls a transcription and/or decoding of the other nucleic acid sequence.

[92] The recombinant vector system of the present invention may be built through various methods known in the art. For example, such detailed methods are disclosed in Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press (2001), the documents of which are hereby incorporated by reference.

[93] The vector of the present invention may be typically built as a vector for cloning or a vector for expression. Also, the vector of the present invention may be built in such a way that a prokaryotic cell or an eukaryotic cell is a host.

[94] For example, if the vector of the present invention is an expression vector and the prokaryotic cell is a host, it is general to comprise powerful promotors capable of carrying out transcription (e.g., tac promotor, lac promotor, lacUV5 promotor, lpp promotor, pLλ promotor, pRλ promotor, rac5 promotor, amp promotor, recA promotor, SP6 promotor, trp promotor, T7 promotor and the like), a ribosome binding site for starting decoding and transcription/decoding termination sequence. If *E.coli* (e.g., HB101, BL21, DH5α, etc.) is used as a host cell, promotor and operator portions of *E. coli* tryptophan biosynthetic pathway (Yanofsky, C., J. Bacteriol., (1984) 158:1018-1024), and a leftward promotor of phage λ (pLλ promotor, Herskowitz, I. and Hagen, D., Ann. Rev. Genet., (1980) 14:399-445) may be used as a regulatory portion. If *Bacillus* sp. is used as a host cell, a promotor of toxin protein gene of *Bacillus* thuringiensis (Appl. Environ. Microbiol. (1998) 64:3932-3938; Mol. Gen. Genet. (1996) 250:734-741) or any promotors expressible in *Bacillus sp.* may be used as a regulatory portion.

[95] Meanwhile, the recombinant vector of the present invention may be prepared by manipulating plasmid (e.g., pCL, pSC101, pGV1106, pACYC177, ColE1, pKT230, pME290, pBR322, pUC8/9, pUC6, pBD9, pHC79, pIJ61, pLAFR1, pHV14, pGEX series, pET series, pUC19 and the like), phage (e.g., λgt4·λB, λ-Charon, λΔz1, M13

and the like) or virus (e.g., SV40, etc.) often used in the art.

[96] Meanwhile, if the vector of the present invention is an expression vector and an eukaryotic cell is a host, promotors derived from a genome of mammal cells (e.g., metallothionein promotor, β-actin promotor, human hemoglobin promotor and human muscle creatin promotor) or promotors derived from mammal virus (e.g., adenoviral late promotor, vaccinia virus 7.5K promotor, SV40 promotor, cytomegalovirus (CMV) promotor, tk promotor of HSV, mouse breast tumor virus (MMTV) promotor, LTR promotor of HIV, promotor of Moloney virus, promotor of Epstein-barr virus (EBV) and promotor of Rous sarcoma virus (RSV)) may be used, wherein they generally have a polyadenylation sequence as a transcription termination sequence. Particularly, the recombinant vector of the present invention comprises a CMV promotor.

[97] The recombinant vector of the present invention may be fused with other sequences in order to facilitate refining of an antibody expressed therefrom. As examples of fused sequences, there are glutathione S-transferase (Pharmacia, USA), maltose binding protein (NEB, USA), FLAG (IBI, USA), 6x His (hexahistidine; Quiagen, USA) and the like. Also, a protein expressed by the vector of the present invention is an antibody, thus the expressed antibody may be easily purified through a protein A column, etc., without an additional sequence for refining.

[98] Meanwhile, the recombinant vector of the present invention comprises an antibiotic resistance gene conventionally used in the art as a selected marker, wherein it may comprise, for example, resistance genes to ampicillin, gentamicin, carbenicillin, chloramphenicol, streptomycin, kanamycin, geneticin, neomycin and tetracycline.

[99] As a vector for expressing the antibody of the present invention, there may be both a vector system, in which a light chain and a heavy chain are simultaneously expressed in one vector, and a system, in which a light chain and a heavy chain are respectively expressed in a separate vector. In the latter case, two vectors may be introduced into a host cell, for example, through co-transformation or targeted transformation. The co-transformation is a method for selecting cells that express both light and heavy chains after simultaneously introducing each vector DNA for coding light and heavy chains into a host cell. The targeted transformation is a method for selecting a cell transformed with a vector comprising a light (or heavy) chain and transforming a selected cell again with a vector comprising a heavy (or light) chain to finally select a cell that expresses both light and heavy chains.

[100] As long as they are capable of stably and continuously cloning and expressing the vector of the present invention, any host cells known in the art may be used, wherein such host cells may comprise *Bacillus* sp. strains such as *Escherichia* coli, *Bacillus* subtilis and *Bacillus* thuringiensis and prokaryotic host cells such as *Streptomyces*, *Pseudomonas* (e.g., *Pseudomonas* putida), *Proteus* mirabilis or *Staphylococcus* (e.g.,

Staphylococcus carnosus), but not limited thereto.

[101] As suitable eukaryotic host cells of the vector, there may be mycetes such as *Aspergillus* species, yeasts such as *Pichia* pastoris, *Saccharomyces* cerevisiae, *Schizosaccharomyces* and *Neurospora* crassa, other lower eukaryotic cells, cells of higher eukaryotes such as insect-derived cells, and cells derived from plants or mammals.

[102] Particularly, host cells may be COS7 cells (monkey kidney cells), NSO cells, SP2/0, Chinese hamster ovary (CHO) cells, W138, baby hamster kidney (BHK) cells, MDCK, myeloma cell lines, HuT 78 cells or 293 cells, more particularly CHO cells, but not limited thereto.

[103] In the present invention, "transformation" and/or "transfection" into host cells may be performed by selecting a suitable standard technology according to host cells as known in the art, comprising any methods for introducing nucleic acid into organisms, cells, tissues or organs. The methods comprise electroporation, plasmogamy, calcium phosphate ($CaPO_4$) precipitation, calcium chloride ($CaCl_2$) precipitation, agitation using silicon carbide fiber, agrobacteria-mediated transformation, PEG, dextran sulfate, lipofectamine, drying/suppression-mediated transformation and the like, but not limited thereto.

[104] In the present invention, the method for producing an antibody or an antigen binding fragment thereof using a host cell may particularly comprise steps of: (a) culturing a host cell transformed with a recombinant vector of the present invention; and (b) expressing an anti-c-Met antibody or an antigen binding fragment thereof in the host cell.

[105] In preparing the antibody above, culturing of a transformed host cell may be performed in an appropriate medium and under culturing conditions known in the art. Such culturing process may be easily adjusted according to a selected strain by those skilled in the art. Such culturing method is disclosed in various documents (e.g., James M. Lee, Biochemical Engineering, Prentice-Hall International Editions, 138-176). Cell culture is divided into suspension culture and attachment culture according to a cell growth type, and batch culture, fed-batch culture and continuous culture according to a culture method. A medium used in culture has to appropriately satisfy requirements of a certain strain.

[106] In culturing of animal cells, the medium comprises various carbon sources, nitrogen sources and microelement ingredients. Examples of usable carbon sources may comprise carbohydrates such as glucose, sucrose, lactose, fructose, maltose, starch and cellulose; fats such as soybean oil, sunflower oil, castor oil and coconut oil; fat acids such as palmitic acid, stearic acid and linoleic acid; alcohols such as glycerol and ethanol; and organic acids such as acetic acid, wherein such carbon sources may be used alone or in combination.

[107] Nitrogen sources, which may be used in the present invention, may comprise, for

example, organic nitrogen sources such as peptone, yeast extract, meat juice, malt extract, corn steep liquor (CSL) and soybean-wheat, and inorganic nitrogen sources such as urea, ammonium sulfate, ammonium chloride, ammonium phosphate, ammonium carbonate and ammonium nitrate, wherein such nitrogen sources may be used alone or in combination. As a phosphorus source, the medium may comprise potassium dihydrogen phosphate, dipotassium hydrogen phosphate and sodium-containing salt corresponding thereto. Also, the medium may comprise metallic salts such as magnesium sulphate or iron sulfate. Besides, the medium may comprise amino acids, vitamins, appropriate precursors and the like.

[108] During culture, compounds such as ammonium hydroxide, potassium hydroxide, ammonia, phosphoric acid and sulfuric acid are added to a culture product in an appropriate way to adjust a pH of the culture product. Also, during culture, bubble formation may be suppressed by using a defoaming agent such as fatty acid polyglycol ester. Also, oxygen or oxygen-containing gas (e.g., air) is injected into a culture product in order to maintain an aerobic state of the culture product. A temperature of the culture product is normally 20℃ to 45℃, preferably 25℃ to 40℃.

[109] The production method may further comprise a step of: (c) collecting an anti-c-Met antibody or an antigen binding fragment thereof expressed in the host cell. An antibody obtained by culturing the transformed host cell may be used in a non-purified state, or further used in a purified state with high purity by using various conventional methods, for example, dialysis, salt precipitation, chromatography and the like. Out of those methods, a method for using chromatography is most often used, wherein a type and order of column may be selected from ion-exchange chromatography, size exclusion chromatography, affinity chromatography, etc., according to antibody characteristics, culture method, etc.

[110]

[111] Another aspect of the present invention provides a composition for detecting c-Met, comprising the antibody or the antigen binding fragment thereof, a kit for detection comprising the same, and a method for detecting c-Met antibody using the same.

[112] The composition for detecting c-Met and the kit comprising the same form an antigen-antibody complex in such a way that an antibody specifically binding to c-Met or an antigen binding fragment thereof comes into contact with a specimen sample, thus effectively detecting c-Met.

[113] As used herein, the term "antigen-antibody complex" means a conjugate between c-Met and an antibody for recognizing the same, in order to identify a tumor or a cancer cell of expressing c-Met in a sample.

[114] A method for quantifying c-Met antigen using a composition for detecting c-Met and using a kit comprising the same may be performed by identifying a formation of an

antigen-antibody complex, wherein identifying of the formation of an antigen-antibody complex may be performed by enzyme immunoassay (ELISA), western blotting, immunofluorescence, immunohistochemistry staining, flow cytometry, immunocytochemistry, radioimmunoassay (RIA), immunoprecipitation assay, immunodiffusion assay, complement fixation assay, a protein chip, etc., but not limited thereto. The ELISA comprises various ELISA methods such as a direct ELISA using a labeled antibody for recognizing an antigen attached to a solid support; an indirect ELISA using a labeled secondary antibody for recognizing a capture antibody in a complex of an antibody for recognizing an antigen attached to a solid support; a direct sandwich ELISA using another labeled antibody for recognizing an antigen in a complex of an antibody and an antigen attached to a solid support; an indirect sandwich ELISA using a labeled secondary antibody for reacting with another antibody for recognizing an antigen in a complex of an antibody and an antigen attached to a solid support and then recognizing such antibody, etc.

[115] As a label for qualitatively or quantitatively making a formation of an antigen-antibody complex measurable, there are an enzyme, a fluorescent material, a ligand, a luminous material, a microparticle, a redox molecule, radio isotope and the like, but not necessarily limited thereto. As the enzymes, there are β-glucuronidase, β-D-glucosidase, β-D-galactosidase, urease, peroxidase, alkaline phosphatase, acetylcholinesterase, glucose oxidase, hexokinase and GDPase, RNase, glucose oxidase and luciferase, phosphofructokinase, phosphoenolpyruvate carboxylase, aspartate aminotransferase, phosphoenolpyruvate decarboxylase, β-lactamase, etc., but not limited thereto.

[116]

[117] Another aspect of the present invention provides a composition for preventing or treating cancer comprising the antibody or the antigen binding fragment thereof of the present invention.

[118] Yet another aspect of the present invention provides a method for preventing or treating cancer, comprising a step of administering a composition comprising the antibody or the antigen binding fragment thereof of the present invention to an individual being in danger of developing cancer or having the same.

[119] Still yet another aspect of the present invention provides a use of cancer treatment and a use of preparing an anticancer drug, with regard to a composition comprising the antibody or the antigen binding fragment thereof of the present invention.

[120] The antibody and the antigen binding fragment thereof are such as that described above.

[121] The antibody or the antigen binding fragment thereof of the present invention is capable of binding to c-Met alone or a combination of c-Met and EGFR with high

affinity to inhibit a growth of cancer cells, such that the antibody alone or in combination with conventional pharmaceutically acceptable carriers can be used in treatment, prevention and diagnosis of hyperproliferative diseases such as cancer.

[122] In the present invention, the term "prevention" means all the acts, which prevent or delay diseases such as cancer, etc., from occurrence or recurrence by an administration of the composition of the present invention, and the term "treatment" means an inhibition of development of diseases such as cancer, reduction of cancer, or removal of cancer.

[123] It may be provided that cancer, a disease applied to the composition of the present invention, is particularly lung cancer, stomach cancer, colon cancer, rectal cancer, triple negative breast cancer (TNBC), glioblastoma, pancreatic cancer, head and neck cancer, breast cancer, ovarian cancer, renal cancer, bladder cancer, prostate cancer, solenoma, salivary gland tumor or thyroid cancer, more particularly lung cancer, stomach cancer, colon cancer, rectal cancer, triple negative breast cancer (TNBC), glioblastoma, pancreatic cancer, head and neck cancer, breast cancer, and much more particularly lung cancer, stomach cancer, colon cancer, rectal cancer, triple negative breast cancer (TNBC), glioblastoma, pancreatic cancer, head and neck cancer, but not limited thereto. In the present invention, it may be provided that cancer is the one caused by, in particular, c-Met overexpression, amplification, mutation or activation, but not limited thereto. In other words, a composition comprising the antibody or the binding fragment thereof of the present invention has an inhibitory effect on proliferation of all the cancerous tumors irrespective of abnormal expression or mutation of c-Met, such that a pharmaceutical use of the present invention is not limited by an expression aspect or presence or absence of mutation of c-Met.

[124] The composition may be a form of a pharmaceutical composition, a quasi-drug composition and a composition for health food.

[125] The composition of the present invention for preventing or treating cancer may further comprise a pharmaceutically acceptable carrier. The pharmaceutically acceptable carrier is the one conventionally used in preparing a formulation, comprising lactose, dextrose, sucrose, sorbitol, mannitol, starch, acacia rubber, calcium phosphate, alginate, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, methylcellulose, methyl hydroxybenzoate, propyl hydroxybenzoate, talc, magnesium stearate, mineral oil and the like, but not limited thereto. Besides the ingredients, the composition of the present invention for preventing or treating cancer may further comprise lubricant, humectant, sweetening agent, flavoring agent, emulsifier, suspending agent, preservative, etc. Suitable pharmaceutically acceptable carriers and preparations are described in detail in Remington's Pharmaceutical Sciences (19th ed., 1995).

[126] The composition of the present invention may be administered orally or parenterally wherein a parenteral administration may be performed by intravenous infusion, subcutaneous infusion, intramuscular injection, intraperitoneal injection, endothelial administration, local administration, intranasal administration, intrapulmonary administration, rectal administration and the like. During an oral administration, protein or peptide is digested, so an oral composition may be formulated in such a way that its active drug is coated or protected from decomposition in stomach. A composition of the present invention may be administered by a predetermined device through which an active substance may be moved into a target cell.

[127] A suitable dosage of the composition of the present invention for preventing or treating cancer varies depending on such factors as a formulation method, an administration type, a patient' age, weight, gender, morbid condition, food, administration time, administration path, excretion speed and response sensitivity, wherein an ordinary skilled doctor may easily determine and prescribe an effective dose for a desired treatment or prevention. According to one exemplary embodiment of the present invention, a daily dose of the pharmaceutical composition of the present invention may amount to 0.001-100 ㎎/㎏ or more. In the present specifications, the term "pharmaceutical effective dose" means an amount enough to treat, prevent and diagnose diseases such as cancer.

[128] The composition of the present invention for preventing or treating cancer may be formulated into a preparation by using pharmaceutically acceptable carriers and/or expedients according to a method, which may be easily performed by those skilled in the art, to which the present invention pertains, such that such composition can be prepared in a mono-dose form or prepared by being inserted into a multi-dose container. At this time, a dosage form may be in a form of solution in oil or aqueous medium, suspension or emulsion, or in a form of extract, powder, suppository, powdered drug, granule, tablet or capsule, and may further comprise a dispersing agent or a stabilizer.

[129] The composition of the present invention may be administered as an individual therapeutic agent or administered in combination with other therapeutic agents, and may be administered sequentially or simultaneously with conventional therapeutic agents.

[130] The antibody or the antigen binding fragment thereof of the present invention may be used in treatment of cancer in such a way that it is injected in vivo in a form of an antibody-therapeutic agent (functional molecule) and a bispecific antibody-therapeutic agent (functional molecule) conjugate, which are such as that described above. Appropriate and desirable various conditions for targeting a drug to a specific target site are reported in documents, for example, Trouet et al., Plenum Press, New York and

London, (1982) 19-30.

[131] According to one specific embodiment of the present invention, as a result of identifying an antitumor activity of the composition of the present invention for preventing or treating cancer in a xenograft mouse model, it was identified that its tumor activity inhibitory efficacy was remarkably excellent compared to the control group (FIGS. 12 and 13).

[132] c-Met, targeted by an antibody or an antigen binding fragment thereof included in the composition of the present invention is a molecule expressed on the surface of cancer cells, thus it may be used in the prevention, treatment and diagnosis of c-Met related cancer in such a way that a functional molecule further is bound to the antibody of the present invention or is administered in combination therewith. The functional molecule may comprise a chemical substance, radioactive nuclide, immunotherapeutic agent, cytokine, chemokine, toxin, biotic agent, enzyme inhibitor and the like.

[133] The functional molecule capable of coupling with the antibody or the fragment thereof of the present invention results in antibody drug-conjugates (ADC) may be a chemical substance, cytokine or chemokine, but not limited thereto. The chemical substance may be, for example, an anticancer drug, particularly, acivicin, aclarubicin, acodazole, acronycine, adozelesin, alanosine, aldesleukin, allopurinol sodium, altretamine, aminoglutethimide, amonafide, ampligen, amsacrine, androgens, anguidine, aphidicolin glycinate, asaley, asparaginase, 5-azacytidine, azathioprine, bacillus calmette-guerin (BCG), Baker's antifol, beta-2-dioxythioguanosine, bisantrene HCl, bleomycin sulfate, bulsufan, buthionine sulfoximine, BWA773U82, BW502U83/HCl, BW 7U85 mesylate, ceracemide, carbetimer, carboplatin, carmustine, chlorambucil, chloroquinoxalin-sulfonamide, chlorozotocin, chromomycin A3, cisplatin, cladribine, corticosteroid, corynebacterium parvum, CPT-11, crisnatol, cyclocytidine, cyclophosphamide, cytarabine, cytembena, dabis maleate, decarbazine, dactinomycin, daunorubicin HCl, deazauridine, dexrazoxane, dianhydro galactitol, diaziquone, dibromodulcitol, didemnin B, diethyldithio carbamate, diglycoaldehyde, dihydro-5-azacytidine, doxorubicin, echinomycin, dedatrexate, edelfosine, eflornithine, Elliot's solution, elsamitrucin, epirubicin, esorubicin, estramustine phosphate, estrogen, etanidazole, ethiophos, etoposide, fadrazole, fazarabine, fenretinide, filgrastim, finasteride, flavone acetic acid, floxuridine, fludarabine phosphate, 5'-fluorouracil, Fluosol™, flutamide, gallium nitrate, gemcitabine, goserelin acetate, hepsulfam, hexamethylene bisacetamide, homoharringtonine, hydrazine sulfate, 4-hydroxyandrostenedione, hydroxyurea, idarubicin HCl, ifosfamide, 4-ipomeanole, iproplatin, isotretinoin, leucovorin calcium, leuprolide acetate, levamisol, liposomal daunorubicin, liposome trapping doxorubicin, lomustine, lonidamine, maytansine, mechlorethamine hydrochloride, melphalan, menogaril, merbarone, 6-mercaptopurine,

mesna, methanol extract of bacillus calmette-guerin, methotrexate, N-methylformamide, mifepristone, mitoguazone, mitomycin-C, mitotane, mitoxantrone hydrochloride, monocyte/macrophage colony-stimulating factor, nabilone, nafoxidine, neocarzinostatin, octreotide acetate, ormaplatin, oxaliplatin, paclitaxel, pala, pentostatin, piperazinedione, pipobroman, pirarubicin, piritrexim, piroxantrone hydrochloride, PIXY-321, plicamycin, porfimer sodium, prednimustine, procarbazine, progestins, pyrazofurin, razoxane, sargramostim, semustine, spirogermanium, spiromustine, streptonigrin, streptozocin, sulofenur, suramin sodium, tamoxifen, taxorere, tegafur, teniposide, terephthalamidine, teroxirone, thioguanine, thiotepa, thymidine injection, tiazofurin, topotecan, toremifene, tretinoin, trifluoperazine hydrochloride, trifluridine, trimetrexate, tumor necrosis factor (TNF), uracil mustard, vinblastin sulfate, vincristine sulfate, vindesine, vinorelbine, vinzolidine, Yoshi 864, zorubicin, cytosine arabinoside, etoposide, melphalan, taxotere, taxol and mixtures thereof, but not limited thereto.

[134]

Mode for the Invention

[135] Hereinafter, the present invention will be described in more detail through Examples. The following Examples are provided only for the purpose of illustrating the present invention in more detail. Thus, according to the purpose of the present invention, it is apparent to those skilled in the art that the Examples are not construed to limit the scope of the present invention.

[136]

[137] **Example 1. Preparation of** hybridoma cell for producing c-Met specific antibody and identification of tumor cell proliferation inhibitory activity thereof

[138]

[139] **(1) Preparation and selection of** hybridoma cell line for producing monoclonal antibody to c-Met protein

[140] A human c-Met Sema domain/Fc fusion protein (self-produced) was intraperitoneally injected as an antigen into a mouse, in order to obtain an immunized mouse needed for developing a hybridoma cell line through animal immunization. Screening was performed through an ELISA analysis method using a human c-Met/His fusion protein as an antigen, in order to select a hybridoma cell specifically responding to c-Met protein only out of a hybridoma cell group.

[141]

[142] **(2) c-Met antibody**

[143] Light chain and heavy chain CDR amino acid sequences of a mouse antibody obtained from a selected hybridoma cell line are shown in Tables 1 and 2 respectively.

[144]

[145] [Table 1]

[146] Hybridoma light chain CDR

Antibody	CDR 1	SEQ ID NOs	CDR 2	SEQ ID NOs	CDR 3	SEQ ID NOs
8C4	GASENIYGALN	1	GATNLAD	2	QNVLSSPYT	3
5G3	SATSSVRYMY	4	DTSNLAS	5	QQWSSYPRT	6

[147] [Table 2]

[148] Hybridoma heavy chain CDR

Antibody	CDR 1	SEQ ID NOs	CDR 2	SEQ ID NOs	CDR 3	SEQ ID NOs
8C4	DYYIN	7	EIFPGSGNTHFSARFKG	8	GDYGFLY	9
5G3	DYTLH	10	YINPYSGYTNYNQKFKD	11	GHMDY	12

[149]

[150] **(3) In vitro tumor cell proliferation inhibitory activity of** hybridoma C-Met antibody

[151] With regard to a c-Met specific mouse antibody obtained from a hybridoma cell line as well as a chimera antibody prepared by fusing the antibody with human heavy chain and light chain constant regions, a tumor cell proliferation inhibitory activity was tested in a human glioblastoma cell line U-87 MG and a human stomach cancer cell line MKN45.

[152]

[153] Particularly, the U-87 MG cells (ATCC, #HTB14) were diluted in a culture medium EMEM (ATCC, #30-2003) containing 10% (v/v) FBS, 100 U / 500 ml penicillin and 100 ㎍ / 500 ml streptomycin (Invitrogen, #15140-122), after which resulting cells were added by 100 ㎕ into each well of a 96-well plate at a concentration of 2.5 X 10^3 cells, such that the plate was cultured under 37℃, 95% RH and 5% (v/v) CO_2 conditions for 18 - 24 hours. The cell culture medium was removed from each well, after which an EMEM medium containing 2% (v/v) FBS was added by 100 ㎕ into each well, and an antibody prepared at 2X of a final concentration (100 nM) was continuously diluted at a ratio of 1/10, such that resulting cells were added by 100 ㎕ into each well at six concentrations (i.e., 200 nM, 20 nM, 2 nM, 200 pM, 20 pM and 2 pM) for each antibody. Then, the plate was cultured for 5 days under 37℃, 95% RH and 5% (v/v) CO_2 conditions, after which resulting cells were fixed with 10% TCA (Trichloroacetic acid; Sigma, #T0699) solution on a final day. The resulting fixed cells were dyed for 25 minutes in such a way that 80 ㎕ of 0.4% SRB (sulforhodamine B)

solution was added into each well, after which resulting cells were washed 5 times with 1% acetic acid solution. Then, 150 ㎕ of 10 mM Tris solution was inserted into each well of a dried plate to dissolve SRB dye, after which its optical density was measured at a wavelength of 540nm by using a microplate reader.

[154] Also, MKN45 (#JCRB0254) cell lines were diluted in an RPMI-1640 medium (Gibco, #A10491) containing 10% (v/v) FBS, after which the resulting cell lines were divided by 2.5 X 10^3 into each well of a 96-well plate, such that the resulting plate was cultured overnight under 37℃, 5% CO_2 conditions. Then, the medium of each well of the plate was replaced with 100 ㎕ of an RPMI-1640 medium containing 1% (v/v) FBS, after which a test antibody was sequentially diluted at a ratio of 1/10 (i.e., 100 nM, 10 nM, 1 nM, 100 pM, 10 pM and 1 pM) to reach 1 pM at a final concentration of 100 nM, such that the resulting antibody was added by 100 ㎕ into each well. Then, the plate was cultured for 5 days under 37℃, 5% CO_2 conditions, after which the medium was removed therefrom, such that a TCA solution was inserted by 200 ㎕ into each well to fix cells. As shown in the test on the U87 MG cell, the cells of the plate were dyed according to a conventional SRB colorimetric assay method, after which an optical density of each well was measured at a wavelength of 540 nm by using a microplate reader. Results of the U87 MG and MKN45 cell lines are shown in Table 3 and FIG. 1.

[155]

[156] [Table 3]

[157] Results of in vitro test on tumor cell proliferation inhibitory activity of hybridoma c-Met antibody

	U-87 MG (GBM, HGF autocrine) IC_{50} (nM)	MKN45 (Gastric cancer, c-Met amplified) IC_{50} (nM)
LY2875358 (Eli Lilly)	> 100	0.34
OA-5D5 (Genentech)	> 100	> 100
hybridoma 8C4	17.5	9.78
hybridoma 5G3	> 100	0.32
8C4 chimera IgG1	32.4	> 100
8C4 chimera IgG2	> 100	12.92
5G3 chimera IgG2	> 100	0.41

[158] As seen in Table 3 and FIG. 1 above, the anti-c-Met 8C4, 5G3 antibodies and chimera antibodies thereof of the present invention all have a tumor cell proliferation inhibitory activity, which is equal to or more excellent than the known c-Met antibodies LY2875358 and OA-5D5 (control group). Thus, the 8C4, 5G3 antibodies and mutants thereof such as chimera antibodies, humanized antibodies and affinity-optimized antibodies to antigen of the present invention may be very valuably used in preventing or treating c-Met related cancer.

[159] Specific consensus sequences for light chain and heavy chain variable regions of the 8C4, 5G3 antibodies of the present invention are shown in the following Table 4.

[160]

[161] [Table 4]

[162] Consensus SEQ ID NOs for light chain and heavy chain variable regions of 8C4, 5G3 antibodies

	Consensus amino acids sequence		Consensus nucleotides sequence	
	light chain	heavy chain	light chain	heavy chain
8C4	DILMTQSPASLSASVGETVTITCGASENIYGALNWYQRKQGKSPQLLIYGATNLADGMSSRFSGSGSGRQFSLKITSLHPDDVATYYCQNVLSSPYTFGGGTKLEIK (SEQ ID NO: 13)	EVQLQQSGAELARPGASVKLSCKASGYTFSDYYINWVKQGTGQGLEWIGEIFPGSGNTHFSARFKGKATLTADKSSSTAYMQLSSLTSTDSAVYFCAGGDYGFLYWGRGTLVTVSA (SEQ ID NO: 15)	gatattctgatgaccca gtctccagcttcactgt ctgcatctgtgggagaa actgtcaccatcacatg tggagcaagtgagaata tttacggtgctttaaat tggtatcagcgaaaaca gggaaaatctcctcagc tcctgatctatggtgca accaacttggcagatgg catgtcatcgaggttca gtggcagtgggtctggt agacagttttctctcaa gatcactagcctgcatc ctgacgatgttgcaacg tattactgtcaaaatgt gctaagtagtccgtaca cgttcggagggggggacc aagctggaaatcaaa (SEQ ID NO: 17)	gaggttcagctgcagca gtctggagctgagctgg cgaggcccggggcttca gtgaagctgtcctgcaa ggcttctggctacacct tcagtgactactatata aactgggtgaagcaggg gactggacagggccttg agtggattggagagatt tttcctggaagtggaaa tactcacttcagtgcga ggttcaagggcaaggcc acactgactgcagacaa atcctccagcacagcct acatgcagctcagcagc ctgacatctacggactc tgcagtctatttctgtg ccgggggtgactacggg tttctttactggggccg agggactctggtcactg tctctgca (SEQ ID

[163]

				NO: 19)
5G3	QIVLTQSPAIMSASPGEKVTMTCSATSSVRYMYWYQQKPGSSPRLLIYDTSNLASGVPGRFSGSGSGTSNSLTISRLEAEDAATYYCQQWSSYPRTFGGGTKLEIK (SEQ ID NO: 14)	QGQLQQSGAELARPGASVKMSCKASGYTFTDYTLHWVKQRPGQGLEWIGYINPYSGYTNYNQKFKDKATLTADKSSSTAYMQLSGLTSEDSAVFYCARGHMDYWGQGTSVTVSS (SEQ ID NO: 16)	caaattgttctcaccagtctccagcaatcatgtctgcatctccaggggagaaggtcaccatgacctgcagtgccacctcaagtgtacgttacatgtactggtaccagcagaagccaggatcctcccccagactcctgatttatgacacatccaacctggcttctggagtccctggtcgcttcagcggcagtgggtctgggacctctaactctctcacaatcagccgattggaggctgaagatgctgccacttattactgccagcagtggagtagttacccacggacgttcggtggaggcaccaagctggaaatcaaa (SEQ ID NO: 18)	cagggccagctgcagcagtctggggctgaactggcaagacctggggcctcagtgaagatgtcctgcaaggcttctggctacacctttactgactacacgctgcactgggtaaaacagaggcctggacagggtctggaatggattggatacattaatccttacagtggttatactaattacaatcagaaattcaaggacaaggccacattgactgcagacaaatcctccagcacagcctacatgcaactgagcggcctgacatctgaagactctgcagtcttttattgtgcaagaggacatatggactactggggtcaaggaacctcagtcaccgtctcctca (SEQ ID NO: 20)

[164] **Example 2. Preparation of humanized antibody of** 8C4 antibody and identification of in vitro tumor cell proliferation inhibitory activity thereof

[165]

[166] As one example, the mouse antibody 8C4 was humanized and an in vitro tumor cell proliferation inhibitory activity thereof was identified, in order to further identify an effect of an antibody prepared in the present invention.

[167]

[168] For a humanized design of 8C4 antibody heavy chains, a human germline gene having a high homology with a gene in a heavy chain variable region of a mouse antibody 8C4 was analyzed first through Ig Blast (http://www.ncbi.nlm.nih.gov/igblast/). In result, it was identified that IGHV3-23 had

48% homology with the 8C4 antibody in an amino acid level, and also identified that IGHV3-11 had 46% homology with the 8C4 antibody in an amino acid level.

[169] The CDR-H1, CDR-H2 and CDR-H3 of the mouse antibody 8C4 was defined by Kabat numbering, and hu8C4-1 was prepared in such a way that the CDR portion of the mouse antibody 8C4 was represented by be introduced into a framework of IGHV3-23. At this time, no. 48 (V→I), no. 49 (S→G), no. 71 (R→A), no. 73 (N→K), no. 78 (L→A) and no. 94 (K→G) amino acids were back-mutated into an original amino acid sequence of the mouse antibody 8C4 to finally build a heavy chain of hu8C4-1. In case of hu8C4-2, the CDR portion of the mouse antibody 8C4 was represented by be introduced into a framework of IGHV3-11, and no. 48 (V→I), no. 49 (S→G), no. 71 (R→A), no. 73 (N→K), no. 78 (L→A) and no. 94 (R→G) amino acids were back-mutated into an original amino acid sequence of the mouse antibody 8C4 to finally build a heavy chain of hu8C4-2.

[170] Even in case of a light chain of 8C4 antibody, for a humanized design, a human germline gene having a high homology with a gene in a light chain variable region of the mouse antibody 8C4 was analyzed through Ig Blast (http://www.ncbi.nlm.nih.gov/igblast/). In result, it was identified that IGKV1-27 had 65.3% homology with the 8C4 antibody in an amino acid level, and that IGKV1-33 had 64.2% homology with the 8C4 antibody in an amino acid level.

[171] The CDR-L1, CDR-L2 and CDR-L3 of the mouse antibody 8C4 were defined by Kabat numbering, and the CRD portion of the mouse antibody 8C4 was represented by be introduced into a framework of IGKV1-33 and a framework of IGKV1-27, thus preparing hu8C4-1 and hu8C4-2 respectively. At this time, amino acid no. 69 (T→R) of both and hu8C4-2 were back-mutated into an original amino acid sequence of the mouse antibody 8C4.

[172]

[173] The 8C4 humanized antibody was expressed in a 293T cell by using a pCLS05 vector (Korea Patent Registration No. 10-1420274). With regard to such obtained humanized antibodies in a form of IgG1, it was identified whether or not they had a tumor cell proliferation inhibitory activity in U-87 MG, a human glioblastoma cell line, by the same method as shown in Example 1 above.

[174] In result, it was identified that the IC_{50} values of hu8C4-1 and hu8C4-2 amounted to 30 nM and 24.6 nM respectively, thus indicating a similar level of anticancer activity to that of a chimera 8C4 antibody (IC_{50}= 32.4 nM).

[175] Specific consensus sequences for light chain and heavy chain variable regions of the hu8C4-1 and hu8C4-2 humanized antibodies are shown in Table 5.

[176]

[177] [Table 5]

[178] Consensus SEQ ID NOs for light chain and heavy chain variable regions of hu8C4-1 and hu8C4-2 humanized antibodies

	Consensus amino acids sequence		Consensus nucleotides sequence	
	light chain	heavy chain	light chain	heavy chain
hu8C4-1	DIQMTQSPSSLSASVGDRVTITCGASENIYGALNWYQQKPGKAPKLLIYGATNLADGVPSRFSGSGSGRDFTFTISSLQPEDIATYYCQNVLSSPYTFGQGTKVEIK (SEQ ID NO: 21)	EVQLVESGGGLVQPGGSLRLSCAASGYTFSDYYINWVRQAPGKGLEWIGEIFPGSGNTHFSARFKGRATLSADKSKNTAYLQMNSLRAEDTAVYYCAGGDYGFLYWGQGTLVTVSS (SEQ ID NO: 23)	gatatccagatgacccagtctcccagcagtctttccgcttctgtgggtgatagggtgacgataacttgcggagcaagtgagaatatttacggtgctttaaattggtaccagcagaagcctgggaaagctccaaagctgctgatctatggtgcaaccaacttggcagatggcgtccctagcaggttcagcggcagtggaagcggcagagacttcactttcacaatctcctccctgcaacccgaggacattgcaacctactattgtcaaaatgtgctaagtagtccgtacacgtttggccaggaaccaaggttgaaattaaa (SEQ ID NO: 25)	gaggttcagttagtggaatccggaggaggactggtgcagcctggtggaagtttgaggctgtcatgcgcagccagtggctacaccttcagtgactactatataaactgggtaagacaggctcccggaaaagggctgagtggattggagagattttcctggaagtggaaatactcacttcagtgcgaggttcaaggccgagccaccctctcagcagacaaaagcaagaataccgcctatctgcagatgaatagccttcgcgcagaagatactgccgtgtattactgtgccgggggtgacttacgggtttctttactggggacagggcaccttggtgacagtctct

[179]

				tct (SEQ ID NO: 27)
hu8C4-2	DIQMTQSPSSLSASVGDRVTITCGASENIYGALNWYQQKPGKVPKLLIYGATNLADGVPSRFSGSGSGRDFTLTISSLQPEDVATYYCQNVLSSPYTFGQGTKVEIK (SEQ ID NO: 22)	QVQLVESGGGLVKPGGSLRLSCAASGYTFSDYYINWIRQAPGKGLEWIGEIFPGSGNTHFSARFKGRATISADKAKNSAYLQMNSLRAEDTAVYYCAGGDYGFLYWGQGTLVTVSS (SEQ ID NO: 24)	gacatccagatgaccagtctccatcctcctgtctgcatctgtaggagacagagtcaccatcacttgcggagcaagtgagaatatttacggtgctttaaattggtatcagcagaaaccagggaaagttcctaagctcctgatctatggtgcaaccaacttggcagatggggtcccatctcggttcagtggcagtggatctgggcgagatttcactctcaccatcagcagcctgcagcctgaagatgttgcaactattactgtcaaaatgtgctaagtagtccgtacacgtttggccaggaaccaaggttgaaattaaa (SEQ ID NO: 26)	caggttcagttagtggaatccggaggaggactggtgaagcctggtggaagtttgaggctgtcatgcgcagccagtggctacaccttcagtgactactatataaacctggatcagacaggctcccggaaaagggctggagtggattggagagattttttcctggaagtggaaatactcacttcagtgcgaggttcaaggccgagccaccatctcagcagacaaagcgaagaatagcgcctatctgcagatgaatagccttcgcgcagaagatactgccgtgtattactgtgccgggggtgactacgggtttctttacttggggacagggcaccttggtgacagtctcttct (SEQ ID NO: 28)

[180] **Example 3. Preparation of humanized antibody of 5G3 antibody and identification of in vitro tumor cell proliferation inhibitory activity thereof**

[181]

[182] Then, the mouse antibody 5G3 of the present invention was humanized to identify an in vitro tumor cell proliferation inhibitory activity thereof.

[183]

[184] Particularly, for a heavy chain design of hu5G3-1, a human germline gene having a highest homology with a gene in a heavy chain variable region of the mouse antibody 5G3 was analyzed first through Ig Blast (http://www.ncbi.nlm.nih.gov/igblast/). In result, it was identified that IGHV1-46 had 67.3% homology with the 5G3 antibody in an amino acid level. The CDR-H1, CDR-H2 and CDR-H3 of the mouse antibody 5G3 were defined by Kabat numbering, and the CRD portion of the mouse antibody 5G3 was represented by be introduced into a framework of IGHV1-46. At this time, amino acid no. 48 (M→I), no. 69 (M→L), no. 71 (R→A), no. 73 (T→K) and no. 78 (V→A) were back-mutated into an original amino acid sequence of the mouse antibody 5G3. By doing so, a heavy chain of hu5G3-1 was built.

[185] For a light chain of hu5G3-1, CDR-grafting was performed in IGKV3-20 gene having 63.5% homology with the 5G3 antibody, and amino acid no. 43 (A→S), no. 60 (D→A) and no. 71 (F→N) were back-mutated to build a light chain of hu5G3-1.

[186] Also, to design a heavy chain of hu5G3-2, the CDR-H1, CDR-H2 and CDR-H3 of the mouse antibody 5G3 defined by Kabat numbering were introduced by using VH3 subtype, which was conventionally known to be most stable. At this time, amino acid no. 67 (F→A), no. 69 (I→L), no. 73 (T→K), no. 90 (Y→F) and no. 94 (T→R) were back-mutated into an original amino acid sequence of the mouse antibody 5G3. By doing so, a heavy chain of hu5G3-2 was built.

[187] For a light chain of hu5G3-2, CDR-grafting was performed in IGVK III gene, which was known to stably form a structure with VH3 subtype, and back-mutation was not performed.

[188]

[189] The 5G3 humanized antibody was expressed in a 293T cell by using a pCLS05 vector (Korea Patent Registration No. 10-1420274). With regard to such obtained humanized antibodies in a form of IgG2, it was identified whether or not they had a tumor cell proliferation inhibitory activity in MKN45, a human stomach cancer cell line, by the same method as shown in Example 1 above.

[190] In result, it was identified that the IC_{50} values of hu5G3-1 and hu5G3-2 amounted to 0.52 nM and 0.5 nM respectively, thus indicating a similar level of anticancer activity to that of a chimera 5G3 antibody (IC_{50} = 0.41 nM).

[191]

[192] Consensus sequences for light chain and heavy chain variable regions of the hu5G3-1 and hu5G3-2 humanized antibodies are shown in Table 6.

[193]

[194] [Table 6]

[195] Consensus SEQ ID NOs for light chain and heavy chain variable regions of hu5G3-1 and hu5G3-2 humanized antibodies

	Consensus amino acids sequence		Consensus nucleotides sequence	
	light chain	heavy chain	light chain	heavy chain
hu5G3-1	EIVLTQSPATLSLSPGERATLSCSATSSVRYMYWYQQKPGQSPRLLIYDTSNLASGIPARFSGSGSGTDNTLTISRLEPEDFAVYYCQQWSSYPRTFGGGTKVEIK (SEQ ID NO: 29)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYTLHWVRQAPGQGLEWIGYINPYSGYTNYNQKFKDRVTLTADKSTSTAYMELSSLRSEDTAVYYCARGHMDYWGQGTLVTVSS (SEQ ID NO: 31)	gaaattgtgttgacacagtctccagccaccctgtctttgtctccaggggaaagagccacctctcctgcagtgccacctcaagtgtacgttacatgtactggtaccagcagaaacctggccagtctcccaggctcctcatctatgacacatccaacctggcttctggcatcccagcaaggttcagtggcagtgggtctgggacagacaactactctcaccatcagcagactggagcctgaagattttgcagtttattactgtcagcagtggagtagttacccacggacgttcggcggagggaccaaggtggagatcaaa (SEQ ID NO: 33)	caggtgcagctggtgcagtctggggctgaggtgaagaagcctggggcctcagtgaaggttcctgcaaggcatctggatacaccttcaccgactacacgctgcactgggtgcgacaggccctggacaagggcttgagtggataggatacattaatccttacagtggttatactaattacaatcagaaattcaaggacagagtcaccttgaccgcagacaaatccacgagcacagcctacatggagctgagcagcctgagatctgaggacacggccgtgtattactgtgctagaggacatatggactactggggccaaggaaccctggtcaccgtctcctca

[196]

				(SEQ ID NO: 35)
hu5G3-2	DIQMTQSPSSLSASVGDRVTITCSATSSVRYMYWYQQKPGKAPKLLIYDTSNLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSSYPRTFGQGTKVEIK (SEQ ID NO: 30)	EVQLVESGGGLVQPGGSLRLSCAASGYTFTDYTLHWVRQAPGKGLEWVGYINPYSGYTNYNQKFKDRATLSADKSKNTAYLQMNSLRAEDTAVFYCARGHMDYWGQGTLVTVSS (SEQ ID NO: 32)	gacatccagatgactcagagtccctcttctctgtctgcctcagtgggagatcgggtcacaatcacatgttcagcaacaagctcagtgcgatacatgtattggtaccagcagaagccaggcaaagccccaaagctgctgatctatgacacatctaatctggccagcggcgtcccatctcgcttctcaggctccggaagcggtactgatttcaccctgactatttctccttgcagcctgaggacttcgcaacctattattgccagcagtggtctagctaccctcgcacattcggccagggaaccaaggtcgaaattaaa (SEQ ID NO: 34)	gaagtccaacttgtggagtcaggaggcgggctcgtgcagccaggcggatcattgcgacttcttgtgctgcctcagggtacaccttcactgattataccttgcattgggttcgccaagcacccggtaagggtctcgaatgggtaggatacattaatccatacagcggctacaccaactacaaccagaaattcaaagacagggctacccttagtgccgacaagtctaagaacaccgcctaccttcagatgaactccttagagccgaggatactgctgtgttttatgcgctaggggtcatatggactactggggacaggggaccttggtgactgtgtcttcc (SEQ ID NO: 36)

[197] **Example 4. Preparation of hinge mutant and testing of tumor cell proliferation inhibitory activity thereof**

[198]

[199] Then, a test on tumor cell proliferation inhibitory activity was performed according to a hinge sequence of human IgG1 heavy chain constant region.

[200]

[201] First of all, a hinge of the human IgG1 heavy chain constant region had an amino acid sequence of "EPKSCDKTHTCPPCP (SEQ ID NO: 37)," which was substituted to

obtain a hinge region mutant having an amino acid sequence of SEQ ID NO: 38 to SEQ ID NO: 44.

[202] The resulting mutants were respectively cloned into a vector comprising the heavy chain variable region of hu8C4-1, hu8C4-2 humanized antibodies prepared in Example 2 above. An in vitro tumor cell proliferation inhibitory activity according to a hinge sequence was identified in U-87 MG by the same method as shown in Example 1 above.

[203] Also, an effect of the 8C4 humanized antibody was analyzed as follows with regard to non-small cell lung cancer cell line NCI-H1993 (ATCC, #CRL-5909). The NCI-H1993 cell lines were diluted in an RPMI-1640 medium (Gibco, #A10491) containing 10% (v/v) FBS, after which resulting cell lines were divided by 3.0 X 10^3 into each well of a 96-well plate, such that the resulting plate was cultured overnight under 37°C, 5% CO_2 conditions. After that, the medium of each well of the plate was replaced with 100 ㎕ of an RPMI-1640 medium containing 2% (v/v) FBS, after which a test antibody was sequentially diluted at a ratio of 1/10 (i.e., 100 nM, 10 nM, 1 nM, 100 pM, 10 pM and 1 pM) to reach 0.001 nM at a final concentration of 100 nM, such that the resulting antibody was added by 100 ㎕ into each well. Then, the plate was cultured for 5 days under 37°C, 5% CO_2 conditions, after which the medium was removed therefrom, such that a TCA solution (Sigma, #T0699) was inserted by 200 ㎕ into each well to fix the cells. Also, the cells of the plate were dyed according to a conventional SRB colorimetric assay method, after which an optical density of each well was measured at a wavelength of 540 nm by using a microplate reader.

[204] Results of hu8C4-1 in U-87 MG and NCI-H1993 (ATCC, #CRL-5909) are shown in Table 7.

[205]

[206] [Table 7]

[207] Hinge region mutant sequences and results of in vitro test on tumor cell proliferation inhibitory activity

SEQ ID NOs	Amino acids	SEQ ID NOs	Nucleotides	U-87 MG (GBM, HGF autocrine) (IC_{50} nM)	NCI-H1993 (NSCLC, c-Met amplified) (IC_{50} nM)
37	EPKSCDKTHTCPPCP	45	gagcccaaatcttgtgacaaaactcacacatgcccaccgtgccca	12.6	> 100
38	ERKCCVECPPCP	46	gagcgaaaatgttgtgtcgagtgcccaccgtgccca	31.0	0.30
39	ECCVECPPCP	47	gagtgttgtgtcgagtgcccaccgtgccca	57.3	> 100
40	ERKCCCPPCP	48	gagcgaaaatgttgttgcccaccgtgccca	37.6	0.23
41	ECCCPPCP	49	gagtgttgttgcccaccgtgccca	25.3	> 100
42	EKCCVECPPCP	50	gagaaatgttgtgtcgagtgcccaccgtgccca	31.4	0.48
43	ERKCCVCPPCP	51	gagcgaaaatgttgtgtctgcccaccgtgccca	30.8	0.47
44	EKCCVCPPCP	52	gagaaatgttgtgtctgcccaccgtgccca	75.9	0.38

[208] As seen in Table 7, there is some difference in the tumor cell proliferation inhibitory activity of the hu8C4 antibody according to a difference of hinge sequence, but it was identified that such antibody effectively inhibited a proliferation of most tumor cells. Accordingly, hereinafter an IgG1 humanized antibody representatively having a hinge region of SEQ ID NO: 38 in hu8C4-1 was named as hu8C4, and an affinity-optimized antibody thereto was prepared to identify an effect thereof.

[209]

[210] **Example 5. Preparation of affinity-optimized antibody of** hu8C4 and identification of in vitro tumor cell proliferation inhibitory activity thereof

[211]

[212] To prepare an affinity-optimized antibody of hu8C4, a phage-displayed scFv library was first prepared by using a phagemid vector displayed in a combined form of scFv and pIII, wherein a schematic structure of the vector is illustrated in FIG. 2. The phagemid vector comprises a scFv fragment of an antibody under a control of an IPTG-inductive lac promotor, wherein a linker sequence used was GGGGS GGGGS GGGGS (SEQ. No. 53).

[213] Then, a mutation-inducing oligonucleotide having an NNK codon was used to introduce variety into the heavy chain and light chain CDR domain of hu8C4. Ac-

cordingly, a hu8C4 scFv library with a fusion of His, HA and pIII was prepared, after which a human c-Met specific antibody was selected from the prepared antibody library.

[214] Particularly, a competitive selection method was used to select an antibody with an improved affinity. A human c-Met antigen was bound according to the manufacturer guidelines in Dynabeads® M-280 (Thermo Fisher Scientific, 11205D). A bead with an antigen binding thereto was blocked for 2 hours by a superblock Tris buffered saline (TBS, Pierce). Also recombinant phage grew overnight at 37°C, and then recombinant phage was centrifuged and a phage of its supernatant was blocked with superblock TBS, 0.05% Tween 20 for 2 hours. Then, the bead was washed with PBS containing 0.05% Twin 20. A blocked phage solution was added into the washed bead, after which the resulting bead was incubated in a rotator for 2 hours for phage binding, such that the resulting bead was washed with PBS containing 0.05% Twin 20. Then, a human c-Met antigen was added into PBS 1 ml containing 0.05% Twin 20, after which the resulting antigen was incubated in a rotator for 24 hours (Rouet R et al. (2012) *Nat* Protoc. 7:364-373). After that, the phage binding to the bead was eluted with 100 mM triethanolamine for 5 minutes, after which an eluent was neutralized with 0.5 M Tris/ Cl (pH 7.2). An eluted phage neutralization liquid was infected with *E. coli* TG1.

[215] An individual clone selected through the experiment grew in a 96-well format of 2xYT broth 200 $\mu\ell$ with added carbenicillin and ampicillin, after which a culture supernatant thereof was directly used for ELISA to select a phage-displayed scFv binding to a plate coated with target protein. Amino acid sequences of light chain and heavy chain CDR regions of a detected antibody are shown in Tables 8 and 9, and the representative amino acid sequences of light chain and heavy chain variable regions of an affinity-optimized antibody are shown in Table 10.

[216]

[217] [Table 8]

[218] List of heavy chain CDR sequences

	List of heavy chain CDR sequences					
	CDR1	SEQ ID NOs	CDR2	SEQ ID NOs	CDR3	SEQ ID NOs
AH01	DYYIN	7	EIDPGSGNTHFSARFKG	54	GDYGFLY	9
AH02	DYYIN	7	EIEPGSGNTHFSARFKG	55	GDYGFLY	9
AH03	DYYIN	7	EIWPGSGNTHFSARFKG	56	GDYGFLY	9
AH04	DYYIN	7	EIYPGSGNTHFSARFKG	57	GDYGFLY	9
AH05	DYYIN	7	EIFPGWGNTHFSARFKG	58	GDYGFLY	9
AH06	DYYIN	7	EIFPGYGNTHFSARFKG	59	GDYGFLY	9
AH07	DYYIN	7	EIFPGSGYTHFSARFKG	60	GDYGFLY	9
AH08	DYYIN	7	EIFPGSGNTWFSARFKG	61	GDYGFLY	9
AH09	DYYIN	7	EIFPGSGNTYFSARFKG	62	GDYGFLY	9
AH12	DYYIN	7	EIFPGWGNTYFSARFKG	63	GDYGFLY	9
AH13	DYYIN	7	EIFPGSGNTHFSARFKG	8	QDYGFLY	64
AH14	DYYIN	7	EIFPGSGNTHFSARFKG	8	EDYGFLY	65
AH15	DYYIN	7	EIFPGSGNTHFSARFKG	8	HDYGFLY	66
AH16	DYYIN	7	EIFPGSGNTHFSARFKG	8	NDYGFLY	67
AH17	DYYIN	7	EIFPGSGNTHFSARFKG	8	VELGFLY	68
AH18	DYYIN	7	EIFPGSGNTHFSARFKG	8	FETGYYL	69
AH19	DYYIN	7	EIFPGSGNTHFSARFKG	8	GEYGYQN	70
AH20	DYYIN	7	EIFPGSGNTHFSARFKG	8	WEYGLSM	71
AH21	DYYIN	7	EIFPHFTSDHFSARFKG	72	GDYGFLY	9
AH22	DYYIN	7	EIFPGSGNTHFSAWMGT	73	GDYGFLY	9
AH23	DYYIN	7	EIFPGSGNESVSFRFKG	74	GDYGFLY	9
AH24	DYYIN	7	EIFPGSGNSAVISRFKG	75	GDYGFLY	9
AH25	DYYIN	7	EIFPGSGNHTVVRRFKG	76	GDYGFLY	9
AH26	DYYIN	7	EIFPGSGNLSMHGRFKG	77	GDYGFLY	9
AH27	DYYIN	7	EIFPGSGNITPVFRFKG	78	GDYGFLY	9
AH28	DYYIN	7	EIFPGSGNPFLTIRFKG	79	GDYGFLY	9
AH29	DYYIN	7	EIFPGSGNSHVVSRFKG	80	GDYGFLY	9
AH30	DYYIN	7	EIFPGSGNLSGIRSFKG	81	GDYGFLY	9
AH31	DYYIN	7	EIFPGSGNFFHGKRFKG	82	GDYGFLY	9
AH32	DYYIN	7	EIFPGSGNPRLGARFKG	83	GDYGFLY	9
AH33	DYYIN	7	EIFPGSGNVSQVERFKG	84	GDYGFLY	9
AH34	DYYIN	7	EIFPGSGNFHGASRFKG	85	GDYGFLY	9
AH35	DYYIN	7	EIFPGSGNVVGGYRFKG	86	GDYGFLY	9
AH36	DYYIN	7	EIFPGSGNPMYDERFKG	87	GDYGFLY	9
AH37	DYYIN	7	EIFPGSGNADLTIRFKG	88	GDYGFLY	9
AH38	DYYIN	7	EIFPGSGNSTNLYRFKG	89	GDYGFLY	9
AH39	DYYIN	7	EIFPGSGNLDIPPRFKG	90	GDYGFLY	9
AH40	DYYIN	7	EIFPGSGNTHFSSAPLP	91	GDYGFLY	9
AH41	DYYIN	7	EIFPGSGNTHFSSEFVS	92	GDYGFLY	9
AH42	DYYIN	7	EIFPGSGNTHFSMSESF	93	GDYGFLY	9
AH43	DYYIN	7	EIFPGSGNTHFSDGSRN	94	GDYGFLY	9
AH44	DYYIN	7	EIFPGSGNTHFSSSVSR	95	GDYGFLY	9
AH45	DYYIN	7	EIFPGSGNTHFSRSVSG	96	GDYGFLY	9
AH46	DYYIN	7	EIFPGSGNTHFSGLSEV	97	GDYGFLY	9
AH47	DYYIN	7	EIFPGSGNTHFSHYWAS	98	GDYGFLY	9
AH48	DYYIN	7	EIFPGSGNTHFSTGLTQ	99	GDYGFLY	9
AH49	DYYIN	7	EIFPGSGNTHFSRHRLH	100	GDYGFLY	9
AH50	DYYIN	7	EIFPGSGNTHFSVPRSM	101	GDYGFLY	9

[219]

	List of heavy chain CDR sequences (continue)					
	CDR1	SEQ ID NOs	CDR2	SEQ ID NOs	CDR3	SEQ ID NOs
AH51	DYYIN	7	EIFPGSGNTHFSLQDYL	102	GDYGFLY	9
AH52	DYYIN	7	EIFPGSGNTHFSDGVSS	103	GDYGFLY	9
AH53	DYYIN	7	EIFPGSGNTHFSMQQGSE	104	GDYGFLY	9
AH54	DYYIN	7	EIFPGSGNTHFSGNVHW	105	GDYGFLY	9
AH55	DYYIN	7	EIFPGSGNTHFSRSPTP	106	GDYGFLY	9
AH56	DYYIN	7	EIFPGSGNTHFSLRMFP	107	GDYGFLY	9
AH57	DYYAN	108	EIFPGSGNTHFSARFKG	8	GDYGFLY	9
AH58	GYYIN	109	EIFPGSGNTHFSARFKG	8	GDYGFLY	9
AH59	QYYIN	110	EIFPGSGNTHFSARFKG	8	GDYGFLY	9
AH60	DQYIN	111	EIFPGSGNTHFSARFKG	8	GDYGFLY	9
AH61	DYYQN	112	EIFPGSGNTHFSARFKG	8	GDYGFLY	9
AH62	DYYIN	7	EIFPGSGNTHFSARFKG	8	GDVGFLY	113
AH63	DYYIN	7	EIFPGSGNTHFSARFKG	8	GDYGFQY	114
AH64	DYYIN	7	EIFPGSGNTHFSARFKG	8	GDYGFLQ	115
AH65	DYYIN	7	EIFPGSGNTHFSARFKG	8	GDQWLLC	116
AH66	DYYIN	7	EIFPGSGNTHFSARFKG	8	WDYGFLY	117
AH67	DYYIN	7	EIFPDSAPSHFSARFKG	118	GDYGFLY	9
AH68	DYYIN	7	EIFPYFLPPHFSARFKG	119	GDYGFLY	9
AH69	DYYIN	7	EIFPGPFTPHFSARFKG	120	GDYGFLY	9
AH70	DYYIN	7	EIFPGSNFGHFSARFKG	121	GDYGFLY	9
AH71	DYYIN	7	EIFPGWGNTHFSARFKG	58	QDYGFLY	64
AH72	DYYIN	7	EIFPGWGNTHFSRSPTP	122	GDYGFLY	9
AH73	DYYIN	7	EIFPGWGNSHVVSRFKG	123	GDYGFLY	9
AH74	DYYIN	7	EIFPGYGNTHFSARFKG	59	QDYGFLY	64
AH75	DYYIN	7	EIFPGYGNTYFSARFKG	124	GDYGFLY	9
AH76	DYYIN	7	EIFPGYGNTHFSRSPTP	125	GDYGFLY	9
AH77	DYYIN	7	EIFPGYGNSHVVSRFKG	126	GDYGFLY	9
AH78	DYYIN	7	EIFPGSGNTYFSARFKG	62	QDYGFLY	64
AH79	DYYIN	7	EIFPGSGNTYFSRSPTP	127	GDYGFLY	9
AH80	DYYIN	7	EIFPGSGNSHVVSRFKG	80	QDYGFLY	64
AH81	DYYIN	7	EIFPGSGNSHVVRSPTP	128	GDYGFLY	9
AH82	DYYIN	7	EIFPGSGNSHVVRSPTP	128	GDYGFLY	9
AH83	DYYIN	7	EIFPGWGNTYFSARFKG	63	QDYGFLY	64
AH84	DYYIN	7	EIFPGWGNTHFSRSPTP	122	QDYGFLY	64
AH85	DYYIN	7	EIFPGWGNSHVVSRFKG	123	QDYGFLY	64
AH86	DYYIN	7	EIFPGYGNTYFSARFKG	124	QDYGFLY	64
AH87	DYYIN	7	EIFPGYGNSHVVSRFKG	126	QDYGFLY	64
AH88	DYYIN	7	EIFPGSGNTHFSRSPTP	106	QDYGFLY	64
AH89	DYYIN	7	EIFPGYGNTHFSRSPTP	125	QDYGFLY	64
AH90	DYYIN	7	EIFPGSGNTYFSRSPTP	127	QDYGFLY	64
AH91	DYYIN	7	EIFPGSGNSHVVRSPTP	128	QDYGFLY	64
AH92	DYYIN	7	EIFPGSGNSHVVSSPTP	129	QDYGFLY	64
AH93	DYYIN	7	EIFPDSAPSYFSARFKG	130	GDYGFLY	9
AH94	DYYIN	7	EIFPGPFTPYFSARFKG	131	GDYGFLY	9
AH95	DYYIN	7	EIFPGSNFGYFSRSPTP	132	GDYGFLY	9
AH96	DYYIN	7	EIFPDSAPSHVVSRFKG	133	GDYGFLY	9
AH97	DYYIN	7	EIFPGPFTSHVVSRFKG	134	GDYGFLY	9
AH98	DYYIN	7	EIFPGSNFSHVVSRFKG	135	GDYGFLY	9
AH99	DYYIN	7	EIFPDSAPSHFSRSPTP	136	GDYGFLY	9
AH100	DYYIN	7	EIFPGPFTPHFSRSPTP	137	GDYGFLY	9

[220]

	List of heavy chain CDR sequences (continue)					
	CDR1	SEQ ID NOs	CDR2	SEQ ID NOs	CDR3	SEQ ID NOs
AH101	DYYIN	7	EIFPGSNFGHFSRSPTP	138	GDYGFLY	9
AH102	DYYIN	7	EIFPDSAPSHVVSSPTP	139	GDYGFLY	9
AH103	DYYIN	7	EIFPGPFTSHVVSSPTP	140	GDYGFLY	9
AH104	DYYIN	7	EIFPGSNFSHVVSSPTP	141	GDYGFLY	9
AH105	QYYIN	110	EIFPDSAPSHFSARFKG	118	GDYGFLY	9
AH106	QYYIN	110	EIFPGPFTPHFSARFKG	120	GDYGFLY	9
AH107	QYYIN	110	EIFPGSNFGHFSARFKG	121	GDYGFLY	9
AH108	DYYIN	7	EIFPDSAPSHFSARFKG	118	QDYGFLY	64
AH109	DYYIN	7	EIFPGPFTPHFSARFKG	120	QDYGFLY	64
AH110	DYYIN	7	EIFPGSNFGHFSARFKG	121	QDYGFLY	64
AH111	DYYIN	7	EIFPDSAPSHFSARFKG	118	GDYGFQY	114
AH112	DYYIN	7	EIFPGPFTPHFSARFKG	120	GDYGFQY	114
AH113	DYYIN	7	EIFPGSNFGHFSARFKG	121	GDYGFQY	114
AH114	DYYIN	7	EIFPDSAPSHFSARFKG	118	GDYGFLQ	115
AH115	DYYIN	7	EIFPGPFTPHFSARFKG	120	GDYGFLQ	115
AH116	DYYIN	7	EIFPGSNFGHFSARFKG	121	GDYGFLQ	115
AH117	DYYIN	7	EIFPGSGNTHFSMSESF	93	HDYGFLY	66
AH118	DYYIN	7	EIFPGSGNTHFSLQDYL	102	HDYGFLY	66
AH119	DYYIN	7	EIFPGSGNTHFSMQGSE	104	HDYGFLY	66

[221]

[222] [Table 9]

[223]

	List of light chain CDR sequences					
	CDR1	SEQ ID NOs	CDR2	SEQ ID NOs	CDR3	SEQ ID NOs
AL01	GASENIYGALN	1	GATNLAD	2	QNVWSSPYT	142
AL02	GASENIYGALN	1	GATNLAD	2	QNVLNSPYT	143
AL03	GASENIYGALN	1	GATNLAD	2	QNVLESPYT	144
AL04	GASENIYGALN	1	GATNLAD	2	QNVLKSPYT	145
AL05	GASENIYGALN	1	GATNLAD	2	QNVLYSPYT	146
AL06	GASENIYGALN	1	GATNLAD	2	QNVLSRPYT	147
AL07	GASENIYGALN	1	GATNLAD	2	QNVLSSPET	148
AL08	GASENIYGALN	1	GATNLAD	2	QNVLSEPYT	149
AL11	GASENIYGALN	1	GATNLAD	2	QNVLESPET	150
AL12	GASENIYGALN	1	GATNLAD	2	QNVLSVPET	151
AL13	GASENIYGALN	1	GATNLAD	2	QNVLSLPET	152
AL14	GASENIYGALN	1	GATNLAD	2	QNVLSIPET	153
AL15	GASENIYGALN	1	GATNLAD	2	QNVLSMPET	154
AL16	GASENIYGALN	1	GATNLAD	2	QNILSSPET	155
AL17	GASENIYGALN	1	GATNLAD	2	QNLISSPET	156
AL18	GASENIYGALN	1	GATNLAD	2	QNMISSPET	157
AL19	GASENIYGALN	1	GATNLAD	2	QNIISLPET	158
AL20	GASENIYGALN	1	GATNLAD	2	QNIISIPET	159
AL21	GASENIYGALN	1	GATNLAD	2	QNSLSSPET	160
AL22	GASENIYGALN	1	GATNLAD	2	QNTLSSPET	161
AL23	GASENIYGALN	1	GATNLAD	2	QNVSSSPET	162
AL24	GASENIYGALN	1	GATNLAD	2	QNVISSPET	163
AL25	GASENIYGALN	1	GATNLAD	2	QNVFSSPET	164
AL26	GASENIYGALN	1	GATNLAD	2	QNVYSSPET	165
AL27	GASENIYGALN	1	GATNLAD	2	QNVRSSPET	166
AL28	GASENIYGALN	1	GATNLAD	2	QNLVSSPET	167
AL29	GASENIYGALN	1	GATNLAD	2	QNLISSPET	156
AL30	GASENIYGALN	1	GATNLAD	2	QNLMSSPET	168
AL31	GASENIYGALN	1	GATNLAD	2	QNIMSSPET	169
AL32	GASENIYGALN	1	GATNLAD	2	QNVHSSPET	170
AL33	GASENIYGALN	1	GATNLAD	2	QNVMSSPET	171
AL34	GASENIYGALN	1	GATNLAD	2	QNLLSSPET	172
AL35	GASENIYGALN	1	GATNLAD	2	QSVLFSPFS	173
AL36	GASENIYGALN	1	GATNLAD	2	QQVLFFPET	174
AL37	GASENIYGALN	1	GATNLAD	2	QNLLSPSFY	175
AL38	GASENIYGALN	1	GATNLAD	2	QSVLFSPFT	176
AL39	GASENIYGALN	1	GATNLAD	2	QNILSSPLF	177
AL40	GASENIYGALN	1	GATNLAD	2	QNTLHYSLV	178
AL41	GASENIYGALN	1	GATNLAD	2	QQVLFFPLL	179
AL42	GASENIYGALN	1	GATNLAD	2	QQVLDFVFY	180
AL43	GASENIYGALN	1	GATNLAD	2	QNVVSSPET	181
AL44	GASENIYGALN	1	DATNLAD	182	QNVLSSPYT	3
AL45	GASENIYGALN	1	FATNLAD	183	QNVLSSPYT	3
AL46	GASENIYGALN	1	HATNLAD	184	QNVLSSPYT	3
AL47	GASENIYGALN	1	KATNLAD	185	QNVLSSPYT	3
AL48	GASENIYGALN	1	PATNLAD	186	QNVLSSPYT	3
AL49	GASENIYGALN	1	QATNLAD	187	QNVLSSPYT	3
AL50	GASENIYGALN	1	SATNLAD	188	QNVLSSPYT	3

[224]

	List of light chain CDR sequences (continue)					
	CDR1	SEQ ID NOs	CDR2	SEQ ID NOs	CDR3	SEQ ID NOs
AL51	GASENIYGALN	1	VATNLAD	189	QNVLSSPYT	3
AL52	GASENIYGALN	1	YATNLAD	190	QNVLSSPYT	3
AL53	GASENIYGALN	1	GATNLAD	2	ITVLSPPYT	191
AL54	GASENIYGALN	1	GATNLAD	2	QNNLVPPFN	192
AL55	GASENIYGALN	1	GATNLAD	2	QHVLFLPYV	193
AL56	GASENIYGALN	1	GATNLAD	2	QAVLTNAYT	194
AL57	GASENIYGALN	1	GATNLAD	2	QNVLRVGYL	195
AL58	GASENIYGALN	1	GATNLAD	2	QSVLRVGYL	196
AL59	GASENIYGALN	1	GATNLAD	2	QNIISSPYT	197
AL60	GASENIYGALN	1	GATNLAD	2	QQVLCESFL	198
AL61	GASENIYGALN	1	GATNLAD	2	QNVLSQSLL	199
AL62	GASENIYGALN	1	GATNLAD	2	QNVLQPSYL	200
AL63	GASENIYGALN	1	GATNLAD	2	QNLLFQPLS	201
AL64	GASENIYGALN	1	GATNLAD	2	QNVLFQPLV	202
AL65	GASENIYGALN	1	GATNLAD	2	QNQLDPSLF	203
AL66	GASENIYGALN	1	GATNLAD	2	MDVLESPYT	204
AL67	GASENIYGALN	1	GATNLAD	2	QALLLSPYT	205
AL68	GASENIYGALN	1	GATNLAD	2	QQLLESPYT	206
AL69	GASENIYGALN	1	GATNLAD	2	NLTLVSPYT	207
AL70	GASENIYGALN	1	GATNLAD	2	GNILDSPYT	208
AL71	GASENIYGALN	1	GATNLAD	2	EQVLLSPYT	209
AL72	GASENIYGALN	1	GATNLAD	2	NNLLDSPYT	210
AL73	GASENIYGALN	1	GATNLAD	2	EEVLSSPYT	211
AL74	GASENIYGALN	1	GATNLAD	2	QNILFVDYT	212
AL75	GASENIYGALN	1	GATNLAD	2	QNVLHLNYT	213
AL76	GASENIYGALN	1	GATNLAD	2	QNVLQTPYT	214
AL77	GASENIYGALN	1	GATNLAD	2	QNILHPGYT	215
AL78	GASENIYGALN	1	GATNLAD	2	QNVLTRGYT	216
AL79	GASENIYGALN	1	GATNLAD	2	ENILYSPYT	217
AL80	GASENIYGALN	1	GATNLAD	2	QNVLGGGQG	218
AL81	GASENIYGALN	1	GATNLAD	2	QNVLEHPLI	219
AL82	GASENIYGALN	1	GATNLAD	2	QNVLDDPFD	220
AL83	GASENIYGALN	1	GATNLAD	2	QNVLDFPLL	221
AL84	GASENIYGALN	1	GATNLAD	2	QNVLYPSLV	222
AL85	GASENIYGALN	1	GATNLAD	2	QNVLFDQQS	223
AL86	GASENIYGALN	1	GATNLAD	2	QNVLSNEET	224
AL87	GASENIYGALN	1	GATNLAD	2	QNVLKHPYT	225
AL88	GASENIYGALN	1	GATNLAD	2	QNVLSPGMW	226
AL89	GASENIYGALN	1	GATGLAD	227	QNVLSSPYT	3
AL90	GASENIYGALN	1	GAQNLAD	228	QNVLSSPYT	3
AL91	GSSRSIYGALN	229	GATNLAD	2	QNVLSSPYT	3
AL92	RAGRSIYGALN	230	GATNLAD	2	QNVLSSPYT	3
AL93	LGRRGIYGALN	231	GATNLAD	2	QNVLSSPYT	3
AL94	EVQVGIYGALN	232	GATNLAD	2	QNVLSSPYT	3
AL95	RPSEKIYGALN	233	GATNLAD	2	QNVLSSPYT	3
AL96	RASAVIYGALN	234	GATNLAD	2	QNVLSSPYT	3
AL97	KTGDLIYGALN	235	GATNLAD	2	QNVLSSPYT	3
AL98	SCRVPIYGALN	236	GATNLAD	2	QNVLSSPYT	3
AL99	VASRGIYGALN	237	GATNLAD	2	QNVLSSPYT	3
AL100	RGRQNIYGALN	238	GATNLAD	2	QNVLSSPYT	3

[225]

	List of light chain CDR sequences (continue)					
	CDR1	SEQ ID NOs	CDR2	SEQ ID NOs	CDR3	SEQ ID NOs
AL101	AAPRGIYGALN	239	GATNLAD	2	QNVLSSPYT	3
AL102	SAPFKIYGALN	240	GATNLAD	2	QNVLSSPYT	3
AL103	LGMDDIYGALN	241	GATNLAD	2	QNVLSSPYT	3
AL104	NVRRGIYGALN	242	GATNLAD	2	QNVLSSPYT	3
AL105	NTSGRIYGALN	243	GATNLAD	2	QNVLSSPYT	3
AL106	LVSRPIYGALN	244	GATNLAD	2	QNVLSSPYT	3
AL107	WTNRPIYGALN	245	GATNLAD	2	QNVLSSPYT	3
AL108	RIPSAIYGALN	246	GATNLAD	2	QNVLSSPYT	3
AL109	GATRGIYGALN	247	GATNLAD	2	QNVLSSPYT	3
AL110	EGGSPIYGALN	248	GATNLAD	2	QNVLSSPYT	3
AL111	GASRGMFRALN	249	GATNLAD	2	QNVLSSPYT	3
AL112	GASGLVFSALN	250	GATNLAD	2	QNVLSSPYT	3
AL113	GASRGTHMALN	251	GATNLAD	2	QNVLSSPYT	3
AL114	GASSRFHNALN	252	GATNLAD	2	QNVLSSPYT	3
AL115	GASRTAFTALN	253	GATNLAD	2	QNVLSSPYT	3
AL116	GASRSTFSALN	254	GATNLAD	2	QNVLSSPYT	3
AL117	GASGPMFDALN	255	GATNLAD	2	QNVLSSPYT	3
AL118	GASHDLYGALN	256	GATNLAD	2	QNVLSSPYT	3
AL119	GASGTLFGALN	257	GATNLAD	2	QNVLSSPYT	3
AL120	GASKAAFGALN	258	GATNLAD	2	QNVLSSPYT	3
AL121	GASEGIVGALN	259	GATNLAD	2	QNVLSSPYT	3
AL122	GASHEIHVALN	260	GATNLAD	2	QNVLSSPYT	3
AL123	GASRGVFGALN	261	GATNLAD	2	QNVLSSPYT	3
AL124	GASGRVRGALN	262	GATNLAD	2	QNVLSSPYT	3
AL125	GASTGSFSALN	263	GATNLAD	2	QNVLSSPYT	3
AL126	GASGNSFDALN	264	GATNLAD	2	QNVLSSPYT	3
AL127	GASEQSYFALN	265	GATNLAD	2	QNVLSSPYT	3
AL128	GASFRQFSALN	266	GATNLAD	2	QNVLSSPYT	3
AL129	GASAPRHSALN	267	GATNLAD	2	QNVLSSPYT	3
AL130	GASMPLFHALN	268	GATNLAD	2	QNVLSSPYT	3
AL131	GASENIYGALN	1	GATNLAD	2	QNILSSPYT	269
AL132	GASENIYGALN	1	GATNLAD	2	QNVLSMPYT	270
AL133	GASENIYGALN	1	GATNLAD	2	QNVLSEPET	271
AL134	GASENIYGALN	1	GATNLAD	2	QNVLYSPET	272
AL135	GASENIYGALN	1	GATNLAD	2	QNVLEEPYT	273
AL136	GASENIYGALN	1	GATNLAD	2	QNVLELPET	274
AL137	GASENIYGALN	1	GATNLAD	2	QNVLEMPET	275
AL138	GASENIYGALN	1	GATNLAD	2	QNILESPET	276
AL139	GASENIYGALN	1	GATNLAD	2	QNVIESPET	277
AL140	GASENIYGALN	1	GATNLAD	2	QNVMESPET	278
AL141	GASENIYGALN	1	GATNLAD	2	QNLLESPET	279
AL142	GASENIYGALN	1	GATNLAD	2	QNVLYEPYT	280
AL143	GASENIYGALN	1	GATNLAD	2	QNILSEPET	281
AL144	GASENIYGALN	1	GATNLAD	2	QNVISEPET	282
AL145	GASENIYGALN	1	GATNLAD	2	QNVMSEPET	283
AL146	GASENIYGALN	1	GATNLAD	2	QNLLSEPET	284
AL147	GASENIYGALN	1	GATNLAD	2	QSVLFEPFS	285
AL148	GASENIYGALN	1	GATNLAD	2	QSVLFEPFT	286
AL149	GASENIYGALN	1	GATNLAD	2	QNILYSPET	287
AL150	GASENIYGALN	1	GATNLAD	2	QNILSLPET	288

[226]

	List of light chain CDR sequences					
	CDR1	SEQ ID NOs	CDR2	SEQ ID NOs	CDR3	SEQ ID NOs
AL151	GASENIYGALN	1	GATNLAD	2	QNILSMPET	289
AL152	GASENIYGALN	1	GATNLAD	2	QNVLYMPET	290
AL153	GASENIYGALN	1	GATNLAD	2	QNVISMPET	291
AL154	GASENIYGALN	1	GATNLAD	2	QNVMSMPET	292
AL155	GASENIYGALN	1	GATNLAD	2	QNLLSMPET	293
AL156	GASENIYGALN	1	GATNLAD	2	QNIISSPET	294
AL157	GASENIYGALN	1	GATNLAD	2	QNVLYLPET	295
AL158	GASENIYGALN	1	GATNLAD	2	QNVIYSPET	296
AL159	GASENIYGALN	1	GATNLAD	2	QNVMYSPET	297
AL160	GASENIYGALN	1	GATNLAD	2	QNLLYSPET	298
AL161	GASENIYGALN	1	GATNLAD	2	QNVISLPET	299
AL162	GASENIYGALN	1	GATNLAD	2	QNVMSLPET	300
AL163	GASENIYGALN	1	GATNLAD	2	QNLLSLPET	301
AL164	RASAVIYGALN	234	GATGLAD	227	QNVLSSPYT	3
AL165	GASENIYGALN	1	GATGLAD	227	QNVLESPYT	144
AL166	GASENIYGALN	1	GATGLAD	227	QNVLSEPYT	149
AL167	GASENIYGALN	1	GATGLAD	227	QNVLSSPET	148
AL168	GASENIYGALN	1	GATGLAD	227	QNVLYSPYT	146
AL169	GASENIYGALN	1	GATGLAD	227	QNILSSPET	155
AL170	GASENIYGALN	1	GATGLAD	227	QNLLSSPET	172
AL171	GASENIYGALN	1	GATGLAD	227	QNVISSPET	163
AL172	GASENIYGALN	1	GATGLAD	227	QNVMSSPET	171
AL173	GASENIYGALN	1	GATGLAD	227	QNVLSLPET	152
AL174	GASENIYGALN	1	GATGLAD	227	QNVLSMPET	154
AL175	GASENIYGALN	1	GATGLAD	227	QSVLFSPFS	173
AL176	GASENIYGALN	1	GATGLAD	227	QNLLFQPLS	201
AL177	GASENIYGALN	1	GATGLAD	227	QQVLFFPLL	179
AL178	GASENIYGALN	1	GATGLAD	227	QSVLFSPFT	176
AL179	RASAVIYGALN	234	GATNLAD	2	QNVLESPYT	144
AL180	RASAVIYGALN	234	GATNLAD	2	QNVLSEPYT	149
AL181	RASAVIYGALN	234	GATNLAD	2	QNVLSSPET	148
AL182	RASAVIYGALN	234	GATNLAD	2	QNVLYSPYT	146
AL183	RASAVIYGALN	234	GATNLAD	2	QNILSSPET	155
AL184	RASAVIYGALN	234	GATNLAD	2	QNLLSSPET	172
AL185	RASAVIYGALN	234	GATNLAD	2	QNVISSPET	163
AL186	RASAVIYGALN	234	GATNLAD	2	QNVMSSPET	171
AL187	RASAVIYGALN	234	GATNLAD	2	QNVLSLPET	152
AL188	RASAVIYGALN	234	GATNLAD	2	QNVLSMPET	154
AL189	RASAVIYGALN	234	GATNLAD	2	QSVLFSPFS	173
AL190	RASAVIYGALN	234	GATNLAD	2	QNLLFQPLS	201
AL191	RASAVIYGALN	234	GATNLAD	2	QQVLFFPLL	179
AL192	RASAVIYGALN	234	GATNLAD	2	QSVLFSPFT	176
AL193	GASRSTFSALN	254	GATNLAD	2	QNVLSIPET	153
AL194	GASMPLFHALN	268	GATNLAD	2	QNVLSIPET	153
AL195	GASRSTFSALN	254	GATNLAD	2	QNVLEEPYT	273
AL196	GASMPLFHALN	268	GATNLAD	2	QNVLEEPYT	273

[227]

[228] [Table 10]

[229] List of sequences of light chain and heavy chain variable regions of affinity-optimized antibody

	Amino acids sequence	SEQ ID NOs
AH71	EVQLVESGGGLVQPGGSLRLSCAASGYTFSDYYINWVRQAPGKGLEW IGEIFPGWGNTHFSARFKGRATLSADKSKNTAYLQMNSLRAEDTAVY YCAGQDYGFLYWGQGTLVTVSS	302
AH72	EVQLVESGGGLVQPGGSLRLSCAASGYTFSDYYINWVRQAPGKGLEW IGEIFPGWGNTHFSRSPTPRATLSADKSKNTAYLQMNSLRAEDTAVY YCAGGDYGFLYWGQGTLVTVSS	303
AH73	EVQLVESGGGLVQPGGSLRLSCAASGYTFSDYYINWVRQAPGKGLEW IGEIFPGWGNSHVVSRFKGRATLSADKSKNTAYLQMNSLRAEDTAVY YCAGGDYGFLYWGQGTLVTVSS	304
AH85	EVQLVESGGGLVQPGGSLRLSCAASGYTFSDYYINWVRQAPGKGLEW IGEIFPGWGNSHVVSRFKGRATLSADKSKNTAYLQMNSLRAEDTAVY YCAGQDYGFLYWGQGTLVTVSS	305
AL130	DIQMTQSPSSLSASVGDRVTITCGASMPLFHALNWYQQKPGKAPKLL IYGATNLADGVPSRFSGSGSGRDFTFTISSLQPEDIATYYCQNVLSS PYTFGQGTKVEIK	306
AL135	DIQMTQSPSSLSASVGDRVTITCGASENIYGALNWYQQKPGKAPKLL IYGATNLADGVPSRFSGSGSGRDFTFTISSLQPEDIATYYCQNVLEE PYTFGQGTKVEIK	307
AL165	DIQMTQSPSSLSASVGDRVTITCGASENIYGALNWYQQKPGKAPKLL IYGATGLADGVPSRFSGSGSGRDFTFTISSLQPEDIATYYCQNVLES PYTFGQGTKVEIK	308
AL166	DIQMTQSPSSLSASVGDRVTITCGASENIYGALNWYQQKPGKAPKLL IYGATGLADGVPSRFSGSGSGRDFTFTISSLQPEDIATYYCQNVLSE	309

[230]

	PYTFGQGTKVEIK	
AL194	DIQMTQSPSSLSASVGDRVTITCGASMPLFHALNWYQQKPGKAPKLL IYGATNLADGVPSRFSGSGSGRDFTFTISSLQPEDIATYYCQNVLSI PETFGQGTKVEIK	310
AL195	DIQMTQSPSSLSASVGDRVTITCGASRSTFSALNWYQQKPGKAPKLL IYGATNLADGVPSRFSGSGSGRDFTFTISSLQPEDIATYYCQNVLEE PYTFGQGTKVEIK	311

[231] Also, an in vitro test on proliferation inhibitory activity was performed on U-87 MG cell line by using a part of the affinity-optimized antibodies, wherein results thereof are shown in Table 11.

[232]

[233] [Table 11]

[234] In vitro tumor cell proliferation inhibitory activity by hu8C4 light chain and heavy chain affinity-optimized antibodies

Antibodies	U-87 MG (GBM, HGF autocrine) Cell proliferation inhibition assay, IC_{50} (nM)		IC_{50} Fold
	affinity-optimized antibodies	hu8C4	
hu8C4 AH71	11.3	95.5	8.5
hu8C4 AH72	10.9	95.5	8.8
hu8C4 AH73	10.9	95.5	8.8
hu8C4 AH85	10.1	95.5	9.5
hu8C4 AL130	5.0	45.0	9.0
hu8C4 AL135	7.1	31.9	4.5
hu8C4 AL165	6.8	39.0	5.7
hu8C4 AL166	9.1	39.0	4.3
hu8C4 AL194	9.6	94.5	9.8
hu8C4 AL195	18.0	94.5	5.3

[235] As seen in Table 11, it was identified that IC_{50} of tumor cell proliferation inhibitory activity of a hu8C4 affinity-optimized antibody in a U-87 MG cell amounted to 5.0 - 18 nM, wherein efficacy thereof was increased 4.3 - 9.8 times more than a parent

antibody hu8C4. The results above represent a test performed on a part of antibodies having an amino acid sequence presented in Tables 8 to 10, wherein an affinity of the parent hu8C4 antibody was optimized and all the antibodies were selected based on an antigen affinity through a selection process. Thus, it is expected that there may be a sufficiently equal effect even with regard to the rest of affinity-optimized antibodies as well as antibodies with a combination of presented heavy chain and light chain variable region CDRs.

[236]

[237] For an additional experiment, 10 kinds of affinity-optimized antibody were prepared by combining the light chain and heavy chain variable regions. A specific combination of light chain and heavy chain sequences are shown in Table 12.

[238]

[239] [Table 12]

[240] List of combined variable region sequences of affinity-optimized antibody

	Heavy chain variable region	Light chain variable region
hu8C4 AH71	AH71(SEQ ID NO: 302)	Light chain variable region of hu8C4-1 antibody (SEQ ID NO: 21)
hu8C4 AH85	AH85(SEQ ID NO: 305)	Light chain variable region of hu8C4-1 antibody (SEQ ID NO: 21)
hu8C4 AL194	Heavy chain variable region of hu8C4-1 antibody (SEQ ID NO: 23)	AL194(SEQ ID NO: 310)
hu8C4 A56	AH85(SEQ ID NO: 305)	AL165(SEQ ID NO: 308)
hu8C4 A62	AH72(SEQ ID NO: 303)	AL130(SEQ ID NO: 306)
hu8C4 A71	AH73(SEQ ID NO: 304)	AL135(SEQ ID NO: 307)
hu8C4 A72	AH73(SEQ ID NO: 304)	AL165(SEQ ID NO: 308)
hu8C4 A73	AH73(SEQ ID NO: 304)	AL166(SEQ ID NO: 309)
hu8C4 A76	AH73(SEQ ID NO: 304)	AL195(SEQ ID NO: 311)
hu8C4 A78	AH71(SEQ ID NO: 302)	AL130(SEQ ID NO: 306)

[241] Then, a tumor cell proliferation inhibitory activity was evaluated by the same method as shown in Example 1 above, wherein results thereof are shown in Table 13 and FIG. 3.

[242]

[243] [Table 13]

[244] In vitro tumor cell proliferation inhibitory activity by affinity-optimized antibody

Antibodies	U-87 MG (GBM, HGF autocrine) Cell proliferation inhibition assay, IC_{50} (nM)		IC_{50} Fold
	Affinity-optimized antibody	hu8C4	
hu8C4 AH71	3.6	49.0	13.6
hu8C4 AH85	3.2	49.0	15.2
hu8C4 AL194	5.3	49.0	9.2
hu8C4 A56	1.7	49.0	28.5
hu8C4 A62	1.8	49.0	27.6
hu8C4 A71	5.0	49.0	9.7
hu8C4 A72	3.6	49.0	13.8
hu8C4 A73	4.0	49.0	12.3
hu8C4 A76	4.3	49.0	11.3
hu8C4 A78	2.6	49.0	18.9

[245] As seen in Table 13 above, it was identified that hu8C4 as well as 10 kinds of key antibody with a combination of light chain and heavy chain variable regions of an affinity-optimized antibody thereof showed a tumor cell proliferation inhibitory activity, too. In particular, IC_{50} of the 10 kinds of antibody amounted to 1.7 - 5.3 nM and it was identified that they had a tumor cell proliferation inhibitory effect, which was 9.2 - 28.5 times more excellent than the parent antibody hu8C4.

[246]

[247] **Example 6. Preparation of** bispecific antibody and in vitro tumor cell proliferation inhibitory activity

[248]

[249] To prepare a bispecific antibody specifically binding to c-Met and EGFR, Erbitux and Vectibix scFv fragments, known to specifically bind to EGFR, were linked respectively to a heavy chain C-terminus of the c-Met antibody of the present invention by a GGGGSGGGGS (SEQ. No. 312) connector.

[250] To increase the stability of the scFv, a 44th residue of a heavy chain and a 100th residue of a light chain were substituted with cystine (Reiter Y. et al., Biochemistry

33(18):5451-5459 (1994)). Erbitux and Vectibix scFv sequences, amino acid sequences of heavy chain of bispecific antibody and a combination of variable regions of bispecific antibody are shown in the following Tables 14 and 15.

[251]

[252] [Table 14]

[253] List of amino acid sequences of EGFR antibody for preparing bispecific antibody as well as bispecific antibody

	Amino acids sequence	SEQ ID NOs
Erbitux scFv HL	QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKCLEWLGVIWSGGNTDYNTPF TSRLSINKDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYEFAYWGQGTLVTVSAGGGGSGG GGSGGGGSGGGGSDILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKY ASESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGCGTKLELK	313
Erbitux scFv LH	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFS GSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGCGTKLELKGGGGSGGGGSGGGGSGGGG SQVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKCLEWLGVIWSGGNTDYNTP FTSRLSINKDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYEFAYWGQGTLVTVSA	314
Vectibix scFv	QVQLQESGPGLVKPSETLSLTCTVSGGSVSSGDYYWTWIRQSPGKCLEWIGHIYYSGNTNYNP SLKSRLTISIDTSKTQFSLKLSSVTAADTAIYYCVRDRVTGAFDIWGQGTMVTVSSGGGGSGG GGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIYD ASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIATYFCQHFDHLPLAFGCGTKVEIK	315
hu8C4 x Erbitux scFv HL heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGYTFSDYYINWVRQAPGKGLEWIGEIFPGSGNTHFSAR FKGRATLSADKSKNTAYLQMNSLRAEDTAVYYCAGGDYGFLYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVERKCCVECPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GKGGGGSGGGGSQVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKCLEWLGVI WSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYEFAYWGQGTL VTVSAGGGGSGGGGSGGGGSGGGGSDILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQ RTNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGCG TKLELK	316
hu8C4 AH71 x Erbitux scFv HL heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGYTFSDYYINWVRQAPGKGLEWIGEIFPGWGNTHFSAR FKGRATLSADKSKNTAYLQMNSLRAEDTAVYYCAGQDYGFLYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVERKCCVECPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GKGGGGSGGGGSQVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKCLEWLGVI WSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYEFAYWGQGTL VTVSAGGGGSGGGGSGGGGSGGGGSDILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQ RTNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGCG TKLELK	317

[254]

	Amino acids sequence (continue)	SEQ ID NOs
hu8C4 AH72 x Erbitux scFv HL heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGYTFSDYYINWVRQAPGKGLEWIGEIFPGWGNTHFSRS PTPRATLSADKSKNTAYLQMNSLRAEDTAVYYCAGGDYGFLYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVERKCCVECPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GKGGGGSGGGGSQVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKCLEWLGVI WSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYEFAYWGQGTL VTVSAGGGGSGGGGSGGGGSGGGGSDILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQ RTNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGCG TKLELK	318
hu8C4 AH73 x Erbitux scFv HL heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGYTFSDYYINWVRQAPGKGLEWIGEIFPGWGNSHVVSR FKGRATLSADKSKNTAYLQMNSLRAEDTAVYYCAGGDYGFLYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVERKCCVECPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GKGGGGSGGGGSQVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKCLEWLGVI WSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYEFAYWGQGTL VTVSAGGGGSGGGGSGGGGSGGGGSDILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQ RTNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGCG TKLELK	319
hu8C4 AH85 x Erbitux scFv HL heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGYTFSDYYINWVRQAPGKGLEWIGEIFPGWGNSHVVSR FKGRATLSADKSKNTAYLQMNSLRAEDTAVYYCAGQDYGFLYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVERKCCVECPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GKGGGGSGGGGSQVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKCLEWLGVI WSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYEFAYWGQGTL VTVSAGGGGSGGGGSGGGGSGGGGSDILLTQSPVILSVSPGERVSFSCRASQSIGTNIIHWYQQ RTNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGCG TKLELK	320

[255]

	Amino acids sequence (continue)	SEQ ID NOs
hu8C4 x Erbitux scFv LH heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGYTFSDYYINWVRQAPGKGLEWIGEIFPGSGNTHFSAR FKGRATLSADKSKNTAYLQMNSLRAEDTAVYYCAGGDYGFLYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVERKCCVECPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GKGGGGSGGGGSDILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYA SESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGCGTKLELKGGGGSGG GGSGGGGSGGGGSQVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKCLEWLGV IWSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYEFAYWGQGT LVTVSA	321
hu8C4 AH71 x Erbitux scFv LH heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGYTFSDYYINWVRQAPGKGLEWIGEIFPGWGNTHFSAR FKGRATLSADKSKNTAYLQMNSLRAEDTAVYYCAQGDYGFLYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVERKCCVECPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GKGGGGSGGGGSDILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYA SESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGCGTKLELKGGGGSGG GGSGGGGSGGGGSQVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKCLEWLGV IWSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYEFAYWGQGT LVTVSA	322
hu8C4 AH72 x Erbitux scFv LH heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGYTFSDYYINWVRQAPGKGLEWIGEIFPGWGNTHFSRS PTPRATLSADKSKNTAYLQMNSLRAEDTAVYYCAGGDYGFLYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVERKCCVECPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GKGGGGSGGGGSDILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYA SESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGCGTKLELKGGGGSGG GGSGGGGSGGGGSQVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKCLEWLGV IWSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYEFAYWGQGT LVTVSA	323

[256]

	Amino acids sequence (continue)	SEQ ID NOs
hu8C4 AH73 x Erbitux scFv LH heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGYTFSDYYINWVRQAPGKGLEWIGEIFPGWGNSHVVSR FKGRATLSADKSKNTAYLQMNSLRAEDTAVYYCAGGDYGFLYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVERKCCVECPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GKGGGGSGGGGSDILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYA SESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGCGTKLELKGGGGSGG GGSGGGGSGGGGSQVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKCLEWLGV IWSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYEFAYWGQGT LVTVSA	324
hu8C4 AH85 x Erbitux scFv LH heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGYTFSDYYINWVRQAPGKGLEWIGEIFPGWGNSHVVSR FKGRATLSADKSKNTAYLQMNSLRAEDTAVYYCAGQDYGFLYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVERKCCVECPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GKGGGGSGGGGSDILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYA SESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGCGTKLELKGGGGSGG GGSGGGGSGGGGSQVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKCLEWLGV IWSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYEFAYWGQGT LVTVSA	325
hu8C4 x Vectibix scFv heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGYTFSDYYINWVRQAPGKGLEWIGEIFPGSGNTHFSAR FKGRATLSADKSKNTAYLQMNSLRAEDTAVYYCAGGDYGFLYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVERKCCVECPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GKGGGGSGGGGSQVQLQESGPGLVKPSETLSLTCTVSGGSVSSGDYYWTWIRQSPGKCLEWIG HIYYSGNTNYNPSLKSRLTISIDTSKTQFSLKLSSVTAADTAIYYCVRDRVTGAFDIWGQGTM VTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQ KPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIATYFCQHFDHLPLAFGCG TKVEIK	326

[257]

	Amino acids sequence (continue)	SEQ ID NOs
hu8C4 AH71 x Vectibix scFv heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGYTFSDYYINWVRQAPGKGLEWIGEIFPGWGNTHFSAR FKGRATLSADKSKNTAYLQMNSLRAEDTAVYYCAGQDYGFLYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVERKCCVECPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GKGGGGSGGGGSQVQLQESGPGLVKPSETLSLTCTVSGGSVSSGDYYWTWIRQSPGKCLEWIG HIYYSGNTNYNPSLKSRLTISIDTSKTQFSLKLSSVTAADTAIYYCVRDRVTGAFDIWGQGTM VTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQ KPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIATYFCQHFDHLPLAFGCG TKVEIK	327
hu8C4 AH72 x Vectibix scFv heavy chain	EVQLVESGGLVQPGGSLRLSCAASGYTFSDYYINWVRQAPGKGLEWIGEIFPGWGNTHFSRS PTPRATLSADKSKNTAYLQMNSLRAEDTAVYYCAGGDYGFLYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVERKCCVECPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GKGGGGSGGGGSQVQLQESGPGLVKPSETLSLTCTVSGGSVSSGDYYWTWIRQSPGKCLEWIG HIYYSGNTNYNPSLKSRLTISIDTSKTQFSLKLSSVTAADTAIYYCVRDRVTGAFDIWGQGTM VTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQ KPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIATYFCQHFDHLPLAFGCG TKVEIK	328
hu8C4 AH73 x Vectibix scFv heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGYTFSDYYINWVRQAPGKGLEWIGEIFPGWGNSHVVSR FKGRATLSADKSKNTAYLQMNSLRAEDTAVYYCAGGDYGFLYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVERKCCVECPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GKGGGGSGGGGSQVQLQESGPGLVKPSETLSLTCTVSGGSVSSGDYYWTWIRQSPGKCLEWIG HIYYSGNTNYNPSLKSRLTISIDTSKTQFSLKLSSVTAADTAIYYCVRDRVTGAFDIWGQGTM VTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQ KPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIATYFCQHFDHLPLAFGCG TKVEIK	329

[258]

	Amino acids sequence (continue)	SEQ ID NOs
hu8C4 AH85 x Vectibix scFv heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGYTFSDYYINWVRQAPGKGLEWIGEIFPGWGNSHVVSR FKGRATLSADKSKNTAYLQMNSLRAEDTAVYYCAGQDYGFLYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVERKCCVECPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GKGGGGSGGGGSQVQLQESGPGLVKPSETLSLTCTVSGGSVSSGDYYWTWIRQSPGKCLEWIG HIYYSGNTNYNPSLKSRLTISIDTSKTQFSLKLSSVTAADTAIYYCVRDRVTGAFDIWGQGTM VTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQ KPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIATYFCQHFDHLPLAFGCG TKVEIK	330

[259] [Table 15]

[260] List of combined variable region sequences of bispecific antibody

	Heavy chain variable region	Light chain variable region
hu8C4 x Erbitux scFv HL	hu8C4 x Erbitux scFv HL (SEQ ID NO: 316)	light chain variable region of hu8C4-1 antibody (SEQ ID NO: 21)
hu8C4 AH71 x Erbitux scFv HL	hu8C4 AH71 x Erbitux scFv HL (SEQ ID NO: 317)	light chain variable region of hu8C4-1 antibody (SEQ ID NO: 21)
hu8C4 AH85 x Erbitux scFv HL	hu8C4 AH85 x Erbitux scFv HL (SEQ ID NO: 320)	light chain variable region of hu8C4-1 antibody (SEQ ID NO: 21)
hu8C4 AL194 x Erbitux scFv HL	hu8C4 x Erbitux scFv HL (SEQ ID NO: 316)	AL194(SEQ ID NO: 310)
hu8C4 A56 x Erbitux scFv HL	hu8C4 AH85 x Erbitux scFv HL (SEQ ID NO: 320)	AL165(SEQ ID NO: 308)
hu8C4 A62 x Erbitux scFv HL	hu8C4 AH72 x Erbitux scFv HL (SEQ ID NO: 318)	AL130(SEQ ID NO: 306)
hu8C4 A71 x Erbitux scFv HL	hu8C4 AH73 x Erbitux scFv HL (SEQ ID NO: 319)	AL135(SEQ ID NO: 307)
hu8C4 A72 x Erbitux scFv HL	hu8C4 AH73 x Erbitux scFv HL (SEQ ID NO: 319)	AL165(SEQ ID NO: 308)
hu8C4 A73 x Erbitux scFv HL	hu8C4 AH73 x Erbitux scFv HL (SEQ ID NO: 319)	AL166(SEQ ID NO: 309)
hu8C4 A76 x Erbitux scFv HL	hu8C4 AH73 x Erbitux scFv HL (SEQ ID NO: 319)	AL195(SEQ ID NO: 311)

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hu8C4 A78 x Erbitux scFv HL	hu8C4 AH71 x Erbitux scFv HL (SEQ ID NO: 317)	AL130(SEQ ID NO: 306)
hu8C4 x Erbitux scFv LH	hu8C4 x Erbitux scFv LH (SEQ ID NO: 321)	light chain variable region of hu8C4-1 antibody (SEQ ID NO: 21)
hu8C4 AH71 x Erbitux scFv LH	hu8C4 AH71 x Erbitux scFv LH (SEQ ID NO: 322)	light chain variable region of hu8C4-1 antibody (SEQ ID NO: 21)
hu8C4 AH85 x Erbitux scFv LH	hu8C4 AH85 x Erbitux scFv LH (SEQ ID NO: 325)	light chain variable region of hu8C4-1 antibody (SEQ ID NO: 21)
hu8C4 AL194 x Erbitux scFv LH	hu8C4 x Erbitux scFv LH (SEQ ID NO: 321)	AL194(SEQ ID NO: 310)
hu8C4 A56 x Erbitux scFv LH	hu8C4 AH85 x Erbitux scFv LH (SEQ ID NO: 325)	AL165(SEQ ID NO: 308)
hu8C4 A62 x Erbitux scFv LH	hu8C4 AH72 x Erbitux scFv LH (SEQ ID NO: 323)	AL130(SEQ ID NO: 306)
hu8C4 A71 x Erbitux scFv LH	hu8C4 AH73 x Erbitux scFv LH (SEQ ID NO: 324)	AL135(SEQ ID NO: 307)
hu8C4 A72 x Erbitux scFv LH	hu8C4 AH73 x Erbitux scFv LH (SEQ ID NO: 324)	AL165(SEQ ID NO: 308)
hu8C4 A73 x Erbitux scFv LH	hu8C4 AH73 x Erbitux scFv LH (SEQ ID NO: 324)	AL166(SEQ ID NO: 309)
hu8C4 A76 x Erbitux scFv LH	hu8C4 AH73 x Erbitux scFv LH (SEQ ID NO: 324)	AL195(SEQ ID NO: 311)
hu8C4 A78 x Erbitux scFv	hu8C4 AH71 x Erbitux scFv	AL130(SEQ ID NO: 306)

[262]

LH	LH (SEQ ID NO: 322)	
hu8C4 x Vectibix scFv	hu8C4 x Vectibix scFv (SEQ ID NO: 326)	light chain variable region of hu8C4-1 antibody (SEQ ID NO: 21)
hu8C4 AH71 x Vectibix scFv	hu8C4 AH71 x Vectibix scFv (SEQ ID NO: 327)	light chain variable region of hu8C4-1 antibody (SEQ ID NO: 21)
hu8C4 AH85 x Vectibix scFv	hu8C4 AH85 x Vectibix scFv (SEQ ID NO: 330)	light chain variable region of hu8C4-1 antibody (SEQ ID NO: 21)
hu8C4 AL194 x Vectibix scFv	hu8C4 x Vectibix scFv (SEQ ID NO: 326)	AL194(SEQ ID NO: 310)
hu8C4 A56 x Vectibix scFv	hu8C4 AH85 x Vectibix scFv (SEQ ID NO: 330)	AL165(SEQ ID NO: 308)
hu8C4 A62 x Vectibix scFv	hu8C4 AH72 x Vectibix scFv (SEQ ID NO: 328)	AL130(SEQ ID NO: 306)
hu8C4 A71 x Vectibix scFv	hu8C4 AH73 x Vectibix scFv (SEQ ID NO: 329)	AL135(SEQ ID NO: 307)
hu8C4 A72 x Vectibix scFv	hu8C4 AH73 x Vectibix scFv (SEQ ID NO: 329)	AL165(SEQ ID NO: 308)
hu8C4 A73 x Vectibix scFv	hu8C4 AH73 x Vectibix scFv (SEQ ID NO: 329)	AL166(SEQ ID NO: 309)
hu8C4 A76 x Vectibix scFv	hu8C4 AH73 x Vectibix scFv (SEQ ID NO: 329)	AL195(SEQ ID NO: 311)
hu8C4 A78 x Vectibix scFv	hu8C4 AH71 x Vectibix scFv (SEQ ID NO: 327)	AL130(SEQ ID NO: 306)

[263] Then, an in vitro anticancer efficacy of a bispecific antibody linking Erbitux and Vectibix scFv fragments was evaluated in a U-87 MG tumor cell line by the same method as shown in Example 1.

[264] Also, a tumor cell proliferation inhibitory activity was evaluated by using NCI-H1993, NCI-H292 and NCI-H820 lung cancer cell lines. Particularly, with regard to an NCI-H1993 (ATCC, #CRL-5909) cell line with c-Met gene overexpressed therein, an NCI-H292 (ATCC, #CRL-1848) cell line with EGFR and c-Met normally expressed

therein, and NCI-H820 (ATCC, #HTB-181) with threonine (T) mutated into methionine (M) in EGFR amino acid no. 790, a tumor cell proliferation inhibitory activity was performed by the following method. Each cell line was diluted in an RPMI-1640 medium (Gibco, #A10491) containing 10% (v/v) FBS, after which the resulting cell lines were divided by 2.0 X 10^3 into each well of a 96-well plate, such that the resulting plate was cultured overnight under 37℃, 5% CO_2 conditions. Then, each well of the plate was replaced with 100 ㎕ of a serum-free medium, after which the resulting plate was cultured under 37℃, 5% CO_2 conditions for 18 hours. After that, the medium was replaced with 100 ㎕ of the RPMI-1640 medium containing 2% (v/v) FBS or HGF 50 ng/ml, after which a test antibody was sequentially diluted at a ratio of 1/10 (i.e., 100 nM, 10 nM, 1 nM, 100 pM, 10 pM and 1 pM) to reach 0.001 nM at a final concentration of 100 nM, such that the resulting antibody was added by 100 ㎕ into each well. Subsequently, the plate was cultured for 5 days under 37℃, 5% CO_2 conditions, after which the medium was removed therefrom, such that a TCA solution was inserted by 200 ㎕ into each well to fix cells. Also, the cells of the plate were dyed according to a conventional SRB colorimetric assay method, after which an optical density of each well was measured at a wavelength of 540 nm by using a microplate reader.

[265] Results of proliferation inhibitory activity in each cell line above are shown in Tables 16 and 17 and FIGS. 4 and 5.

[266]

[267] [Table 16]

[268] In vitro tumor cell proliferation inhibitory activity by bispecific antibody

Bispecific antibodies	Cell proliferation inhibition assay, IC_{50} (nM)	
	U-87 MG (GBM, HGF autocrine)	NCI-H1993 (NSCLC, c-Met amplified)
hu8C4 x Vectibix scFv	0.06	0.32
hu8C4 AH71 x Erbitux scFv HL	0.06	0.41
hu8C4 AH85 x Erbitux scFv HL	0.06	0.48
hu8C4 AL194 x Erbitux scFv HL	0.07	0.64
hu8C4 A56 x Erbitux scFv HL	0.07	0.57
hu8C4 A62 x Erbitux scFv HL	0.08	0.65
hu8C4 A70 x Erbitux scFv HL	0.07	0.67
hu8C4 A72 x Erbitux scFv HL	0.06	0.49
hu8C4 A73 x Erbitux scFv HL	0.06	0.50
hu8C4 A76 x Erbitux scFv HL	0.06	0.49
hu8C4 A78 x Erbitux scFv HL	0.06	0.76

[269] [Table 17]

[270] In vitro lung cancer cell line proliferation inhibitory activity by bispecific antibody

Bispecific antibodies	Cell proliferation inhibition assay, IC_{50} (nM)			
	NCI-H292 (NSCLC)		NCI-H820 (NSCLC : EGFR T790M, c-Met amplified)	
	no HGF	HGF 50ng/ml	no HGF	HGF 50ng/ml
hu8C4 x Vectibix scFv	0.70	0.24	> 100	4.2
hu8C4 AH71 x Erbitux scFv HL	0.51	0.22	> 100	8.5
hu8C4 AH85 x Erbitux scFv HL	0.43	0.23	> 100	7.6
hu8C4 AL194 x Erbitux scFv HL	0.41	0.24	> 100	19.0
hu8C4 A56 x Erbitux scFv HL	0.42	0.29	> 100	21.7
hu8C4 A62 x Erbitux scFv HL	0.74	0.28	> 100	40.2
hu8C4 A70 x Erbitux scFv HL	0.74	0.23	> 100	40.9
hu8C4 A72 x Erbitux scFv HL	0.78	0.23	> 100	19.5
hu8C4 A73 x Erbitux scFv HL	0.87	0.26	> 100	38.4
hu8C4 A76 x Erbitux scFv HL	0.73	0.21	> 100	10.3

[271] In result, there was no difference in efficacy between bispecific antibodies prepared from U-87 MG tumor cell line by the method and it was identified that an activity inhibitory efficacy thereof was about 15 times more excellent than IC_{50} of hu8C4 optimized antibody. Also, as a result of evaluating a tumor cell proliferation inhibitory activity using NCI-H1993, NCI-H292 and NCI-H820 lung cancer cell lines, it was

identified that there was no difference in efficacy between bispecific antibodies prepared.

[272] Such the results suggest that the antibody of the present invention has a proliferation inhibitory effect on all the cancer types regardless of an overexpression or mutation of c-Met and EGFR, thus may be effectively used in these cancer types.

[273]

[274] **Example 7. Comparative evaluation of in vitro tumor cell proliferation inhibitory activity of** bispecific antibody compared to combined therapy

[275]

[276] Eight types of cancer were used to compare a tumor cell proliferation inhibitory activity between a combined therapy of each antibody targeting c-Met and EGFR respectively and the bispecific antibody of the present invention.

[277] Particularly, a tumor cell proliferation inhibitory activity was evaluated in a lung cancer cell line NCI-H292 (ATCC, #CRL-1848), an HGF-autocrinal glioblastoma cell line U-87 MG (ATCC, #HTB-14), lung cancer cell lines NCI-H1648 (ATCC #CRL-5882) and NCI-H596 (ATCC #HTB-178), HCC827 (ATCC, #CRL2868), a colon cancer cell line LS174T (ATCC, #CL-188), a triple negative breast cancer (TNBC) cell line BT20 (ATCC, #HTB-19) and a pancreatic cancer cell line KP4 (JCRB, #RCB1005). The NCI-H1648 cell line is characterized by a normal expression of EGFR and c-Met, the NCI-H596 cell line is characterized by a deletion of some sequence of exon no. 14 of MET gene, and the HCC827 cell line is characterized by a deletion of some sequence of exon no. 19 of EGFR gene. Also, the LS174T cell line has a KRAS mutation and the KP4 is characterized by autocrining HGF.

[278] The U-87 MG cell line was evaluated by a method of Example 1 and the NCI-H292 cell line was evaluated by a method of Example 6. Also, the NCI-H1648, NCI-H596 and HCC827 cell lines were diluted in an RPMI-1640 medium (Gibco, #A10491) containing 10% (v/v) FBS, after which the resulting cell lines were divided by 2.0 X 10^3 in each well of a 96-well plate. The LS174T cell line was diluted in a DMEM medium (Gibco, #11995-065) containing 10% (v/v) FBS, after which the resulting cell lines were divided by 2.0 X 10^3. The BT20 cell line was diluted in an EMEM medium (ATCC, #30-2003) containing 10% (v/v) FBS, after which the resulting cell lines were divided by 3.0 X 10^3. And, the KP4 cell line was diluted in an RPMI-1640 medium (Gibco, #A10491) containing 10% (v/v) FBS, after which the resulting cell lines were divided by 1.5 X 10^3, such that the resulting plate was cultured overnight under 37°C, 5% CO_2 conditions. Then, each well of the plate was replaced with 100 $\mu\ell$ of a serum-free medium, after which the resulting plate was cultured under 37°C, 5% CO_2 conditions for 18 hours. After that, the medium was replaced with 100 $\mu\ell$ of the RPMI-1640 medium containing 2% (v/v) FBS or HGF 50 ng/ml, after which a test antibody

was sequentially diluted at a ratio of 1/10 (i.e., 100 nM, 10 nM, 1 nM, 100 pM, 10 pM and 1 pM) to reach 1 pM at a final concentration of 100 nM, such that the resulting antibody was added by 100 ㎕ into each well. Then, the plate was incubated for 5 days under 37℃, 5% CO_2 conditions, after which the medium was removed therefrom, such that a TCA solution was inserted by 200 ㎕ into each well to fix cells. Also, the cells of the plate were dyed according to a conventional SRB colorimetric assay method, after which an optical density of each well was measured at a wavelength of 540 nm by using a microplate reader.

[279]

[280] Results of this Example are shown in Tables 18 to 21 and FIGS. 6 to 8.

[281]

[282] [Table 18]

[283] Comparative evaluation of in vitro tumor cell proliferation inhibitory activity between combined therapy and bispecific antibody in U-87 MG and NCI-H292 cell lines

Antibodies	Cell proliferation inhibition assay, IC_{50} (nM)		
	U-87 MG (GBM, HGF autocrine)	NCI-H292 (NSCLC)	
		No HGF	HGF 50 ng/ml
Vectibix	>100	0.09	>100
hu8C4	83.9	>100	>100
hu8C4 + Vectibix combined	79.0	0.10	0.34
hu8C4 x Vectibix scFv	0.4	0.15	0.12
C-EM1-MAb	> 100	5.29	5.73
C-LA480	858.8	-	-
C-OA-5D5	171.9	-	-
C-AbF46	> 100	-	-

[284] [Table 19]

[285] Comparative evaluation of in vitro tumor cell proliferation inhibitory activity between combined therapy and bispecific antibody in NCI-H1648 and NCI-H596 cell lines

Antibodies	Cell proliferation inhibition assay, IC_{50} (nM)			
	NCI-H1648 (NSCLC)		NCI-H596 (NSCLC, c-Met mutated)	
	No HGF	HGF 50ng/ml	No HGF	HGF 50ng/ml
Vectibix	> 100	> 100	> 100	> 100
hu8C4	> 100	> 100	> 100	2.3
hu8C4 + Vectibix combined	> 100	> 100	> 100	2.4
hu8C4 x Vectibix scFv	15.4	29.5	> 100	0.4

[286] [Table 20]

[287] Comparative evaluation of in vitro tumor cell proliferation inhibitory activity between combined therapy and bispecific antibody in LS174T, BT20 and KP4 cell lines

Antibodies	Cell proliferation inhibition assay, IC_{50} (nM)		
	LS174T (Colon, KRAS G12V)	BT20 (TNBC)	KP4 (Pancreas)
	HGF 50ng/ml	HGF 50ng/ml	HGF autocrine
Vectibix	> 100	> 100	> 100
hu8C4	> 100	> 100	42.0
hu8C4 + Vectibix combined	34.4	> 100	36.4
hu8C4 x Vectibix scFv	33.4	~ 100	27.0
C-EM1-MAb	-	> 100	> 100

[288] [Table 21]

[289] Comparative evaluation of in vitro tumor cell proliferation inhibitory activity between combined therapy and bispecific antibody in HCC827 and NCI-H596 cell lines

Antibodies	Cell proliferation inhibition assay, IC_{50} (nM)		
	HCC827 (NSCLC, EGFR mutated)		NCI-H596 (NSCLC, c-Met mutated)
	No HGF	HGF 50 ng/ml	HGF 50 ng/ml
Tarceva	2.96	> 100	> 100
Vectibix	> 100	> 100	> 100
hu8C4	> 100	> 100	67.2
hu8C4 x Vectibix scFv	> 100	> 100	0.8
LA480	> 100	> 100	> 100
INC280	> 100	> 100	42.5
EMD1214063	> 100	> 100	68.2
Xalkori	-	-	87.3
Tarceva + hu8C4 combined	3.24	3.09	-
Tarceva + hu8C4 x Vectibix scFv combined	2.35	2.42	-
Tarceva + LA480 combined	3.24	4.78	-
Tarceva + INC280 combined	3.06	2.88	-
Tarceva + EMD1214063 combined	2.80	4.10	-

[290] In result, it was identified that a tumor cell proliferation inhibitory capacity of the bispecific antibody of the present invention was more excellent than that of hu8C4, Vectibix or a combined therapy of two antibodies in the 8 kinds of tumor cell line all. Also, it was identified that it had a remarkably excellent tumor cell proliferation inhibitory capacity in U-87MG, NCI-H292, BT20 and KP4 cell lines when compared to EM1-MAb (Janssen) used as a control bispecific antibody.

[291] Moreover, it was identified that both hu8C4 and hu8C4 x Vectibix scFv had an excellent tumor cell proliferation inhibitory capacity compared to a control antibody, when compared to LA480 (Lilly), OA-5D5 (Genentech) and AbF46 (Samsung), which were c-Met target antibodies in U-87MG cell lines.

[292] Also, Tarceva, an EGFR tyrosine kinase inhibitor in HCC827 cell line, showed re-

sistance under HGF processing conditions, but it was identified that it showed an excellent tumor cell proliferation inhibitory capacity when being processed in combination with Tarceva, hu8C4, hu8C4 x Vectibix scFv or c-Met inhibitors under such conditions.

[293] Also, as a result of comparing various EGFR inhibitors and c-Met inhibitors in NCI-H596 cell line, it was identified that a tumor cell proliferation inhibitory capacity of hu8C4 x Vectibix scFv was excellent compared to EGFR or c-Met single target drug.

[294]

[295] **Example 8. Measurement of binding capacity to** ECD (BIAcore)

[296]

[297] Then, to measure a binding capacity of the c-Met antibody of the present invention to an extracellular domain (ECD), binding of c-Met antibody and bispecific antibody to c-Met ECD and EGFR ECD was measured between human and cynomolgus monkey by using BIAcore.

[298] Particularly, a human c-Met ECD (ACROBiosystems, MET-H5227), a cynomolgus monkey c-Met ECD (SiNo. Biological, 90304-C08H), a human EGFR ECD strep (ACROBiosystems, EGR-H5285) and a cynomolgus monkey EGFR ECD (SiNo. Biological, 90285-C08B) were used.

[299] First of all, to capture an anti-c-Met antibody and a bispecific antibody, an Fc-specific anti-human IgG antibody (SouthernBiotech, 2047-01) was fixed to a CM5 sensor chip in the level of 10000 RU. The antibodies were diluted in HBS-EP buffer (0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA and 0.005% (v/v) Surfactant P20) at a concentration of 1 - 2 μg/ml, after which the resulting antibodies were injected into a CM5 chip with an anti-human Ig Fc fixed thereto at a flow rate of 30 $\mu\ell$/min for 10 - 120 seconds, and then captured in a range of 150 - 200 RU. Each antigen was used after being diluted at 10, 5, 2.5, 1.25, 0.625, 0.3125 and 0.15625 nM, after which the resulting antigens were sequentially injected from a lower concentration. Then, the resulting antigens were injected at a flow rate of 30 $\mu\ell$/min for 5 minutes to carry out binding, after which a running buffer was injected thereinto for 10 - 15 minutes to carry out a dissociation. 15 $\mu\ell$ of 10 mM Glycine-HCl (pH 1.5) was used to revive the chip. A binding and dissociation speed for each cycle was evaluated by using a "1:1 Langmuir binding" model in BIAevaluation software version 4.1, and biacore data are summarized in Tables 22 and 23.

[300]

[301] [Table 22]

[302] Measurement of affinity to c-Met ECD

hu8C4	Binding constant (k_{on}, 1/Ms)	Dissociation constant (k_{off}, 1/s)	Affinity to antigen (K_D, M)
Human c-Met	6.77×10^{5}	2.148×10^{-4}	3.173×10^{-10}
Cynomolgus monkey c-Met	7.467×10^{5}	3.447×10^{-4}	4.616×10^{-10}
hu8C4 AH71	Binding constant (k_{on}, 1/Ms)	Dissociation constant (k_{off}, 1/s)	Affinity to antigen (K_D, M)
Human c-Met	8.306×10^{5}	8.301×10^{-5}	9.993×10^{-11}
Cynomolgus monkey c-Met	-	-	-
hu8C4 x Vectibix scFv	Binding constant (k_{on}, 1/Ms)	Dissociation constant (k_{off}, 1/s)	Affinity to antigen (K_D, M)
Human c-Met	7.339×10^{5}	2.041×10^{-4}	2.78×10^{-10}
Cynomolgus monkey c-Met	7.77×10^{5}	3.37×10^{-4}	4.338×10^{-10}

[303] [Table 23]

[304] Measurement of affinity to EGFR ECD

Vectibix	Binding constant (k_{on}, 1/Ms)	Dissociation constant (k_{off}, 1/s)	Affinity to antigen (K_D, M)
Human EGFR	5.278×10^{5}	1.5×10^{-4}	2.841×10^{-10}
Cynomolgus monkey EGFR	9.37×10^{5}	1.963×10^{-4}	2.095×10^{-10}
hu8C4 x Vectibix scFv	Binding constant (k_{on}, 1/Ms)	Dissociation constant (k_{off}, 1/s)	Affinity to antigen (K_D, M)
Human EGFR	7.776×10^{4}	1.257×10^{-4}	1.617×10^{-9}
Cynomolgus monkey EGFR	1.424×10^{5}	1.274×10^{-4}	8.942×10^{-10}

[305] The data were used to prove that the hu8C4, hu8C4 x Vectibix scFv bispecific antibodies of the present invention bind to c-Met ECD of human and cynomolgus monkey with an excellent affinity.

[306]

[307] **Example 9. Measurement of c-Met antibody binding capacity to c-Met ECD, EGFR ECD between various animal species (ELISA)**

[308]

[309] Binding of c-Met antibody and bispecific antibody to c-Met ECD and EGFR ECD between mouse, cynomolgus monkey and human was measured by using ELISA.

[310] Particularly, mouse c-Met (SiNo. Biological Inc, 50622-M08H), cynomolgus monkey c-Met (SiNo. Biological Inc, 90304-C08H), human c-Met (R&D Systems, 358-MT), mouse EGFR (SiNo. Biological Inc, 51091-M08H), cynomolgus monkey EGFR (SiNo. Biological, 90285-C08B) and human EGFR (Abcam, 155639) antigens were all divided into a 96-well plate at a concentration of 2 μg/ml, after which the resulting plate was reacted at 4°C overnight. After being blocked at room temperature for 1 hour, hu8C4 x Vectibix scFv bispecific antibody was sequentially diluted at a ratio of 1/5 from 100 nM to measure its binding capacity in 7 concentration sections (i.e., 100 nM, 20 nM, 4 nM, 800 pM, 160 pM, 32 pM and 6.4 pM).

[311] After binding the hu8C4 x Vectibix scFv bispecific antibody at room temperature for 1 hour, anti-human IgG, $F(ab')_2$ fragment specific-HRP conjugated antibody (Jackson Immunoresearch, 109-035-097) was diluted at a ratio of 1 : 2500, after which the resulting antibody was reacted at room temperature for 1 hour. Color development was made by using TMB (Sigma, T4444) solution, wherein its value was measured at an optical density of 450 nm and its ELISA results are shown in FIG. 9.

[312] In result, it was identified that hu8C4 monospecific antibody and hu8C4 x Vectibix scFv bispecific antibody did not bind to a mouse c-Met and a mouse EGFR, but bind to monkey and human c-Mets and EGFRs. Also, it was identified that a human IgG antibody, used as a negative control group, did not bind at all. The results above suggest that the c-Met antibody of the present invention is specific only to human and monkey c-Mets and EGFRs.

[313]

[314] **Example 10. Cross-reactivity of c-Met antibody to various receptors on the surface of cells**

[315]

[316] Specificity of hu8C4 antibody specifically binding to c-Met according to the present invention as well as its cross-reactivity to other receptor tyrosine kinase antigens were analyzed by an indirect ELISA method, and 5 antigens of FGF R3, VEGFR R2, IGF IR, PDGF R and RON were selected out of key receptor tyrosine kinases to perform an

analysis.

[317] In this Example, human c-Met Fc chimera (R&D systems, 358-MT_CF), human FGF R3 (IIIc) Fc chimera (R&D systems, 766-FR), human IGF-I R (R&D systems, 391-GR-050), human PDGF Rβ Fc chimera (R&D systems, 385-PR_CF), human VEGF R2 Fc chimera (R&D systems, 357-KD_CF) and human MSP R/Ron (R&D systems, 1947-MS-050) were used as an antigen.

[318] Each antigen was diluted in 0.05 M carbonate-bicarbonate (Sigma, C3041) buffer at a concentration of 1 ㎍/ml, after which the resulting antigen was added into each well of a 96-well plate (Corning, #2592), such that the resulting plate was coated at 4℃ overnight. The plate was washed once with TBS-T, after which TBS-T containing 4% - skim milk was added by 200 ㎕ into each well of the resulting plate in order to inhibit a non-specific binding, such that the resulting plate was reacted at 37℃ for 1 hour. Then, the plate was washed once with TBS-T buffer, after which a primary antibody was sequentially diluted in TBS-T buffer containing 2% - skim milk from a highest concentration of 30 nM to 0.002 nM, such that the resulting antibody was added by 100 ㎕ into each well, thus being reacted at 37℃ for 2 hours. After being washed three times with TBS-T buffer, an anti-human kappa light chains-peroxidase (Sigma, A7164) was diluted at a ratio of 1 : 5000 as a secondary antibody, after which the resulting antibody was added by 100 ㎕ into each well, thus being reacted at 37℃ for 1 hour. Then, after being washed three times with TBS-T buffer, TMB solution (Sigma, T4444) was added by 100 ㎕ into each well to carry out an color developing reaction, after which 2 N ammonium sulfate solution was added by 50 ㎕ into each well to stop the reaction. An optical density was measured based on a value at a wavelength of 450 nm by using a microplate reader and a reference wavelength of 570 nm was used. A degree of binding of an anti-c-Met antibody to each antigen was proportionate to an optical density signal value, wherein results thereof are shown in Table 24.

[319]

[320] [Table 24]

[321] Binding specificity of anti-c-Met antibody hu8C4 to various antigens

	hu8C4 binding (A_{450nm} - A_{570nm})											
Ab.conc. (nM)	c-Met		IGF-IR		RON		PDGFR		VEGFR2		FGFR3	
30.000	2.55	2.51	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.01	0.01	0.02
6.000	1.96	2.03	0.00	0.00	0.00	0.00	-0.01	-0.01	-0.01	-0.01	0.00	0.01
1.200	1.81	1.74	0.00	0.00	0.00	0.00	-0.01	-0.01	-0.01	-0.01	0.00	0.01
0.240	1.48	1.54	0.00	0.00	0.00	0.00	-0.01	-0.01	-0.02	-0.02	-0.01	0.00
0.048	0.76	0.76	0.00	0.00	0.00	0.00	-0.01	-0.01	-0.02	-0.01	0.00	0.00
0.010	0.21	0.20	0.00	0.00	0.00	0.00	-0.01	-0.01	-0.01	-0.01	0.00	0.00
0.002	0.05	0.05	0.00	0.00	0.00	0.00	-0.01	-0.01	-0.01	-0.01	0.00	0.00
Blank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

[322] As seen in Table 24, the hu8C4 antibody of the present invention preferentially binds to c-Met, and it was identified that it did hardly bind to other antigens of FGF R3, VEGFR R2, IGF IR, PDGF R and RON.

[323]

[324] **Example 11. In vitro internalization activity of c-Met antibody and c-Met level inhibitory activity of** bispecific antibody

[325]

[326] It was identified that the c-Met antibody of the present invention had an in vitro internalization activity in tumor cells as well as an effect on reducing a receptor level by a bispecific antibody capable of simultaneously binding to c-Met and EGFR.

[327] First of all, an antibody internalization occurs by a physiological activity of a normal receptor, wherein, when binding to a specific ligand, the receptor normally expressed outside cells becomes activated through a homo- or hetero- dimerization and causes a receptor-mediated endocytosis. An antibody specific to a receptor of a cell has a capacity to induce such phenomenon and is internalized into the cell by causing the endocytosis, thus inducing a decomposition of the receptor, reducing a degree of expression thereof, and possibly inhibiting a signal transduction by a certain receptor. An amount of antibodies bound outside cells may be detected by using a fluorescence-activated cell sorting (FACS) device, thus finding an amount of antibodies internalized inside the cells. In case of binding antibodies by using an antibody with FITC binding to an anti-human kappa LC as a secondary antibody for a light chain of an antibody to be measured, it is possible to quantitatively measure an amount of antibodies, which are not internalized, but remain binding to a target receptor outside cells, thus

identifying an amount of internalized antibodies accordingly. It is possible to measure a background signal by a non-specific binding of an antibody used in a test by using a human IgG antibody, non-specific to an antigen, thus measuring a fluorescent signal by an actual specific binding.

[328] In this Example, a MKN45 cell line (#JCRB0254), which was a stomach cancer cell line, was used to identify an in vitro internalization activity of c-Met antibody inside tumor cells. MKN45 expresses a c-Met receptor at a high level by amplification of MET gene, such that a phosphorylation of the c-Met receptor is induced in an HGF-nondependent way. A test was performed as follows to see if a c-Met receptor is internalized into a cell by an anti-c-Met antibody hu8C4, thus reducing a level of expression.

[329] First of all, MKN45 stomach cancer cell lines were divided by 5.0 x 10^5 into each well of a 6-well plate containing an RPMI-1640 medium (2 ml) containing 10% (v/v) FBS, after which the plate was cultured under 37℃, RH 95% and 5% CO_2 conditions for 24 hours. An anti-c-Met antibody to be analyzed as well as an anti-IgG antibody (control group) were diluted to reach a final concentration of 100 nM, after which the resulting antibodies were reacted overnight. As a plate to be used as a non-internalized control group was treated as an anti-c-Met antibody and a human IgG antibody (control group), after which the resulting plate was reacted at 4℃ for 1 hour. Then, cells of each well were collected with 1 ml of an enzyme-free cell dissociation buffer (Gibco, #13151), after which the collected cells were washed twice with a cold PBS. As a secondary antibody, anti-human kappa LC-FITC (LSBio #LS-C60539) was diluted at a ratio of 1 : 2000, after which the resulting antibody was added thereinto, thus being reacted at 4℃ for 1 hour. Then, the cells were washed twice with PBS, after which the resulting cells were fixed with 100 ㎕ of BD Cytofix (BD, #554655) and washed once with PBS, such that an FITC geo-mean (MFI) value, a degree of fluorescent staining, was measured by using a BD FACS Canto II parenchymatous cell analyzer. An amount of antibodies bound outside cells was obtained by a following formula, wherein results thereof are shown in Table 25.

[330]

[331] Surface bound Ab(%) = $[(MFI_{[37℃\ exp.]} - MFI_{[IgG\ control]}) / (MFI_{[4℃\ control]} - MFI_{[IgG\ control]})] \times 100$

[332]

[333] [Table 25]

[334] Measurement of internalization of hu8C4 and OA-5D5 control antibodies to MKN45 stomach cancer cell line

Antibody	OA-5D5	hu8C4
FITC MFI [IgG control]	127	127
FITC MFI [4℃ control]	1763	1444
FITC MFI [37℃ exp.]	1724	858
Surface bound Ab(%)	98	56

[335] As seen in Table 25 above, it can be shown that OA-5D5, an anti-c-Met antibody used as a control group, was hardly internalized into cells, while the hu8C4 antibody of the present invention was internalized about 40% or more into cells in MKN45 stomach cancer cell line. That is, it is shown that the hu8C4 antibody remarkably reduces a level of expression of a c-Met receptor.

[336] Then, a test for measuring a receptor level on NCI-H820 lung cancer cell line was performed in order to identify an effect of reducing a receptor level by a bispecific antibody capable of simultaneously binding to c-Met receptor and EGFR receptor. The NCI-H820 cell line is a cell line suitable for measuring an effect of reducing a receptor level by an anti-c-Met x EGFR bispecific antibody, because a c-Met receptor was expressed in a level of about 83,000 SABC (specific antibody-binding capacity) and an EGFR receptor is expressed in a level of about 74,000 SABC.

[337]

[338] First of all, NCI-H820 cell lines were divided by 1.0 x 10^5 into each well of a 6-well plate with an RPMI-1640 medium (2 ml) containing 10% (v/v) FBS, after which the resulting plate was cultured overnight under 37℃, RH 95% and 5% CO_2 conditions for 24 hours. Then, it was replaced with a serum-free medium, after which the resulting plate was cultured overnight under 37℃, RH 95% and 5% CO_2 conditions for 24 hours. Then, an anti-c-Met antibody, an anti-c-Met x EGFR bispecific antibody, an anti-EGFR antibody and a human IgG antibody as a control group, which were to be analyzed, were diluted and treated in a medium containing 2% - FBS to reach a final concentration of 10 nM, after which the resulting antibodies were cultured for 5 days. After that, cells of each well were collected with 1 ml of an enzyme-free cell dis-sociation buffer, after which the collected cells were washed twice with a cold PBS. Subsequently, goat F(ab`)$_2$ anti-mouse IgG-CSF (R&D Systems Cat.#F0103B) was added by 10 ㎕ into each well as a secondary antibody, thus being reacted at 4℃ for 1

hour. Then, the cells were washed twice with PBS, after which the resulting cells were fixed with 100 ㎕ of BD Cytofix (BD, #554655) and washed once with PBS, such that an FITC geo-mean (MFI) value, a degree of fluorescent staining, was measured by using a BD FACS Canto II parenchymatous cell analyzer.

[339] In result, when treating an anti-c-Met antibody hu8C4, an EGFR receptor was hardly decreased, but a c-Met receptor was remarkably decreased to a level of 2% (FIG. 10). Also, an anti-EGFR antibody Vectibix reduced the EGFR receptor to a level of about 83%, but a c-Met receptor was hardly decreased. By contrast, in case of treating the hu8C4 x Vectibix bispecific antibody of the present invention simultaneously binding to c-Met and EGFR receptors, it was identified that the EGFR receptor was decreased to a level of about 21% and the c-Met receptor was decreased to a level of about 4%, respectively.

[340] Thus, it was identified that the hu8C4 x Vectibix bispecific antibody of the present invention remarkably reduced a level of expression of c-Met and EGFR receptors simultaneously.

[341]

[342] **Example 12. Identification of c-Met and** EGFR in vitro signal inhibitory activity of bispecific antibody

[343]

[344] Then, an experiment using an NCI-H820 cell line was performed to identify an effect of the bispecific antibody of the present invention on the activity of antigen and signal transduction materials.

[345]

[346] First of all, NCI-H820 cell lines were divided into a 6-well plate at a concentration of 5 x 10^5 cells per well, after which the resulting plate was cultured overnight under 37°C, 5% CO_2 conditions, such that it was replaced with a serum-free medium and cultured overnight again. An antibody was diluted and treated in a serum-free medium at a concentration of 100 nM, after which the resulting antibody was reacted for 24 hours, such that HGF (Gibco, PHG0254) and EGF (R&D Systems, 236-EG-200) were treated at a concentration of 50 ng/ml and 10 ng/ml respectively 15 minutes before collecting cells. Then, the cells were dissolved in a dissolution buffer to carry out a collection of cells, after which a protein concentration was quantified by using a Lowry assay method. 20 ㎍ of protein was loaded onto each well and run in SDS-PAGE, after which blotting was performed in a nitrocellulose membrane. After blocking the membrane, all the primary antibodies were diluted and reacted at a ratio of 1 : 1,000, after which HRP-binding anti-rabbit antibody was diluted at a ratio of 1 : 5,000 and reacted as secondary cells. Then, the antibodies absorbed onto the membrane were reacted with enhanced chemiluminescence (ECL), after which the resulting antibodies

were measured by using an LC-3000 device.

[347] In result, as seen in FIG. 11, when treating hu8C4 x Vectibix scFv bispecific antibody, the EGFR phosphorylation, Erk phosphorylation and Akt phosphorylation were remarkably decreased more than treating hu8C4 or Vectibix antibody alone.

[348] Thus, the hu8C4 x Vectibix scFv bispecific antibody of the present invention may reduce an activity of receptor such as EGFR, Erk, Akt, etc., and downstream signal transduction substances in NCI-H820 cell line. In result, it is shown that the antibody of the present invention shows an efficacy through a signal transduction inhibition.

[349]

[350] **Example 13. Identification of tumor cell proliferation inhibitory activity in U-87 MG xeno**graft mouse model

[351]

[352] An experiment was performed representatively by using hu8C4 IgG2 x Vectibix scFv in order to identify a tumor cell proliferation inhibitory activity by the bispecific antibody of the present invention in an HGF-dependent U-87 MG cell xenograft model.

[353]

[354] First of all, human glioblastoma U-87 MG cell lines were cultured under 37°C, 5% CO_2 conditions by using an EMEM (ATCC® 30-2003™) medium containing L-glutamine (300 ㎎/ℓ), 25 mM HEPES, 25 mM $NaHCO_3$, 10 % heat inactivated FBS and the like. Then, U-87 MG cells were subcutaneously inoculated by 200 ㎕ into a flank of a 6 to 8 week-old male athymic nude mouse (Harlan) at a concentration of 1 x 10^7 per mouse. After identifying that a tumor volume formed in 25 days after inoculation reached 60 - 130 mm^3, a grouping was performed, after which a test material was intraperitoneally administered once a week for 4 weeks (total 5 times: 0, 7, 14, 21 and 28 days). The test material was administered 5 mg/kg, and a tumor volume and a mouse weight were measured twice a week. For data, a comparison between an excipient control group and a test material-administered group was generally verified by using Student t-test, and a statistical method used was Origin Pro 8.5 program. "Maximum inhibition %" indicates an inhibition % of tumor growth compared to a solvent-treated control group.

[355] In result, a group administered with 3.5 mg/kg and 6.8 mg/kg of hu8C4 IgG2 x Vectibix scFv had a maximum inhibition 96% for a tumor volume compared to a solvent control group, and a group administered with 1.5 mg/kg thereof had a maximum inhibition 80%, thus reducing a tumor volume to a significant level from a 7th day after administration until the final day of the test ($p < 0.01$) (FIG. 12). Also, when compared to BsAB-01 as a positive control group, the bispecific antibody of the present invention reduced a tumor growth to a significant level ($p < 0.01$).

[356] Thus, it was identified from results above that the bispecific antibody of the present invention remarkably reduced a tumor growth, thus having an excellent antitumor efficacy.

[357]

[358] **Example 14. Identification of tumor cell proliferation inhibitory activity in NCI-H820 xenograft mouse model**

[359]

[360] NCI-H820 cell line, which is a cell line with threonine (T) of EGFR amino acid no. 790 mutated into methionine (M) and with a MET gene amplified, is known as a resistant cell line of AZD9291 (osimertinib, tagrisso), which is a third generation EGFR TKI (Darren A. E. Cross, et al., Cancer Discov. 4(9): 1046-1061 (2014)). An evaluation was made in an NCI-H820 xenograft mouse model by representatively using hu8C4 x Vectibix scFv out of the bispecific antibodies of the present invention, in order to see a tumor cell proliferation inhibitory activity of the bispecific antibody in NCI-H820 cell line having resistance to such EGFR TKI.

[361] Particularly, a mouse used in this Example was a 6-week-old male mouse (Jackson Laboratory, STOCK Hgftm1.1 (HGF) Aveo Prkdcscid/J), wherein a mouse HGF gene was removed therefrom and transformed to express a human HGF gene. The NCI-H820 (ATCC, #HTB-181) cell line was inserted into a flask for cell culture along with an RPMI1640 medium containing 10% FBS, after which the resulting flask was cultured under 37°C, 5% CO_2 conditions. Then, the resulting cells were washed with PBS and 2.5% trypsin-EDTA (Gibco, 15090) was diluted 10 times, after which it was added thereinto to separate the cells. After that, a centrifugation (1,000 rpm, 5 min.) was performed to get rid of supernatant and obtain a cell suspension in a new medium. Subsequently, a cell viability was identified by a microscope, after which the resulting cells were diluted in a serum-free medium at a concentration of 5.0 x 10^7 cells/ml, thus preparing cell lines. The cell lines prepared were subcutaneously administered into a mouse by an amount of 0.1 ml/head. After administration, when a tumor size in a region with cell lines transplanted thereinto reached about 100 - 150 mm^3, cell lines were distributed so that a tumor size of each group can be evenly dispersed according to a ranked tumor size. Then, oncogenesis was identified twice a week from a 7th day after starting cell administration until 28th day after a day of grouping (day of starting an administration of test material) and after closing an administration of test material, after which a tumor's major axis and minor axis were measured by a calipers, thus calculating a tumor size ($ab^2/2$ (a: a length of major axis, b: a length of minor axis)). Statistical analysis was performed by Prism 5.03 (GraphPad Software Inc., San Diego, CA, USA). If a p value is less than 0.05, it was judged as statistically significant.

[362] In result, in all the groups administered with hu8C4 x Vectibix scFv from a 4th day

after starting an administration of test material until 28th day thereof, it was shown that a tumor proliferation inhibitory activity was significantly higher than a solvent control group ($p<0.001$), and it was also identified that a tumor inhibition ratio amounted to maximum 100% (FIG. 13). On the other hand, AZD9291 (Selleckchem), used as a positive control group, did not show a significant difference from the solvent control group.

[363]

[364] **Example 15. Identification of in vitro tumor cell proliferation inhibitory activity by a combined administration of 5G3 c-Met antibody and HER2 antibody**

[365]

[366] An in vitro test on cell proliferation inhibitory activity was performed by NCI-H2170 cell line, in order to evaluate a tumor cell proliferation inhibitory activity according to a combination of the anti-c-Met antibody 5G3 of the present invention and anti-HER2 antibody. NCI-H2170 cell line (ATCC #CRL-5928) is a non-small cell lung cancer (NSCLC) tumor cell line, wherein, as a result of measuring its receptor level, EGFR was expressed in the level of about 2,700 specific antibody-binding capacity (SABC), while c-Met was expressed in the level of about 11,000 SABC.

[367] Particularly, NCI-H2170 cells were diluted in an RPMI-1640 culture medium containing 10% (v/v) FBS, after which the resulting cells were added by 100 $\mu\ell$ into a plate at a concentration of 3.0×10^3 cells per well, such that the resulting plate was cultured under 37°C, 95% RH and 5% (v/v) CO_2 conditions for 18 - 24 hours. Then, the cell culture medium of each well was removed therefrom, after which an RPMI-1640 medium containing 2% (v/v) FBS was added by 100 $\mu\ell$ into each well. After that, antibodies prepared at 2X of a final concentration (100 nM) were continuously diluted at a ratio of 1/10, such that the resulting antibodies were added by 100 $\mu\ell$ into each well at six concentrations (i.e., 200 nM, 20 nM, 2 nM, 200 pM, 20 pM and 2 pM) for each antibody. The plate was cultured for 5 days under 37°C, 95% RH and 5% (v/v) CO_2 conditions, after which 20 $\mu\ell$ of WST-8 solution (CCK-8, Dojindo) was added into each well on the final day to carry out color development for 1 - 2 hours, such that an optical density was measured at a wavelength of 450 nm by a microplate reader.

[368] Results of cell proliferation inhibitory activity are shown in Table 26 and FIG. 14.

[369]

[370] [Table 26]

[371] In vitro tumor cell proliferation inhibitory activity by a combined therapy of anti-c-Met antibody and anti-HER2 antibody

Antibodies	Cell proliferation inhibition assay, IC_{50} (nM)	
	NCI-H2170 (NSCLC)	
	No HGF	HGF 50ng/ml
A091-F1	> 100	> 100
5G3	> 100	> 100
A091-F1 + 5G3 combined	> 100	11.22

[372] As seen in Table 26, it was identified that a combined treatment of 5G3 and A091 antibody (Korea Patent Registration No. 10-1515535) as an anti-HER2 antibody had a more excellent tumor cell proliferation inhibitory capacity than a single treatment of each antibody in NCI-H2170 tumor cell line.

[373]

[374] **Example 16. Identification of in** vivo tumor cell proliferation inhibitory activity by a combined administration of 5G3 c-Met antibody and HER2 antibody in an NCI-H2170 xenograft mouse model as a human lung cancer cell line

[375]

[376] An anticancer activity experiment was performed on an NCI-H2170 xenograft mouse model as a lung cancer cell line, in order to see a combined efficacy of HER2 antibody and c-Met antibody.

[377] Particularly, in this Example a tumor size of a mouse was measured by the same method as shown in Example 14 by using the same mouse as shown in Example 13 above. Results of evaluating an antitumor efficacy by a combination of A091 and 5G3 in an NCI-H2170 xenograft mouse model as a lung tumor cell are shown in FIG. 15.

[378] In result, in case of carrying out a single administration of A091 alone or a combined administration of A091 and 5G3, a tumor volume was decreased to a significant level compared to a solvent control group from a 14th day after administration ($p < 0.05$). Also, a group administered with a combination of A091 and 5G3 showed a significant decrease in a tumor volume compared to a group administered with A091 alone or a group administered with BsAB02 (US2010/0254988 A1) as a control bispecific antibody ($p < 0.01$).

[379]

[380] **Example 17. Identification of tumor cell proliferation inhibitory activity in NCI-H596 xeno**graft mouse model

[381]

[382] As NCI-H596 cell line was a lung cancer cell line with a mutation in exon14 of c-

Met, an evaluation was made on an NCI-H596 xenograft mouse model, in order to identify an anticancer effect of hu8C4 x Vectibix scFv.

[383] In this Example, a tumor size of a mouse was measured by using the same mouse and the same method as shown in Example 14 above.

[384] Results of evaluating an anticancer efficacy after administering hu8C4 x Vectibix scFv once or twice a week for total 4 weeks in an NCI-H596 xenograft model as a lung tumor cell are shown in FIG. 16.

[385] As a result of measuring a tumor size, a level of tumor size in a group administered with hu8C4 x Vectibix scFv 10 mg/kg twice a week showed a statistically significant difference compared to a control group from an 11th day after starting an administration of test material until the end of an experiment, and levels of tumor sizes in a group administered with hu8C4 x Vectibix scFv 5 mg/kg twice a week and a group administered with hu8C4 x Vectibix scFv 10 mg/kg once a week were also significantly lower compared to a control group from an 18th day after starting an administration of test material. Also, a level of tumor size in a group administered with test material had a tendency of change in a dose-correlated way according to a test material dose, and a tumor size of a test group was lower compared to a control group even after a final day of administering a test material (Day 28).

[386]

[387] **Example 18. Identification of tumor cell proliferation inhibitory activity in** EBC-1 xenograft mouse model

[388]

[389] As EBC-1 was a lung cancer cell line with an amplification of c-Met gene, an evaluation was made on an EBC-1 xenograft mouse model, in order to identify an anticancer effect of hu8C4 x Vectibix scFv.

[390] A mouse used in this Example was a six-week-old female athymic nude mouse (Harlan). EBC-1 (JCRB, #JCRB0820) cell lines were inserted into a flask for cell culture together with an EMEM medium containing 10% FBS, after which the resulting cell lines were cultured under 37°C, 5% CO_2 conditions. Cell lines were prepared in such a way that the resulting cell lines were diluted in a serum-free medium at a concentration of 5.0 x 10^7 cells/ml, after which the cell lines were subcutaneously administered into a mouse by an amount of 0.1 ml/head. When a tumor size in a region with cell lines transplanted thereinto reached about 100 - 150 mm^3, hu8C4 x Vectibix scFv was administered once or twice a week for total 4 weeks, after which a tumor size of the mouse was measured by the same method as shown in Example 14.

[391] Results of evaluating an anticancer efficacy by hu8C4 x Vectibix scFv in an EBC-1 xenograft model as a lung cancer cell are shown in FIG. 17.

[392] As a result of measuring a tumor size, a level of tumor size in a group administered

with hu8C4 x Vectibix scFv 10 mg/kg twice a week showed a statistically significant difference compared to a control group from a 7th day after starting an administration of test material until a 56th day after starting an administration of test material. A group administered with hu8C4 x Vectibix scFv 5 mg/kg twice a week and a group administered with the same once a week showed a significant low level compared to a control group from an 18th day after starting an administration of test material. Also, a level of tumor size in a group administered with test material had a tendency of change in a dose-correlated way according to a test material dose, and a level of tumor size in a group administered with hu8C4 x Vectibix scFv 10 mg/kg twice a week during an observation period after a final day (Day 28) of administering a test material was significantly low compared to a control group until a 56th day after starting an administration of test material. In particular, it was found that one individual in a group administered with hu8C4 x Vectibix scFv 10 mg/kg twice a week had a complete response on an 18th day after starting an administration of test material.

[393]

[394] **Example 19. Effect of reducing c-Met and** EGFR on the surface of cancer cells by bispecific antibody

[395]

[396] An effect of reducing c-Met and EGFR on the surface of in vitro tumor cells by the bispecific antibody (hu8C4 x Vectibix scFv) of the present invention was identified and compared with an effect of the c-Met antibody (hu8C4) of the present invention, vectibix, c-Met/EGFR combination, and other antibodies.

[397] A receptor generally located on a cell membrane was internalized into a cell when binding to an antibody, thus an amount thereof located on the cell membrane was decreased. A decrease in the receptor on such cell membrane causes an inhibition of receptor activation and a decrease in a downstream signal thereof by a ligand binding.

[398] In this Example, a lung adenocarcinoma cell line HCC827 was used to observe a decrease in c-Met and EGFR on a cell membrane. HCC827 has an EGFR E746-A750 deletion mutation and overexpresses c-Met. HCC827 was treated with the bispecific antibody (hu8C4 x Vectibix scFv) of the present invention and other antibodies, after which immunofluorescence staining was performed by an antibody specific to c-Met and EGFR, such that the resulting cell line was analyzed with a fluorescence activated cell sorter, thus measuring an amount of c-Met and EGFR on the surface of cells. A detailed method is as follows.

[399] First of all, HCC827 cells (ATCC® CRL-2868™) were divided by 3.0 x 10^5 into each well of a 6-well plate containing an RPMI-1640 medium (2 ml) containing 10% (v/v) FBS, after which the plate was cultured under 37°C, RH 95% and 5% CO_2 conditions for 24 hours. The bispecific antibody (hu8C4 x Vectibix scFv) of the

present invention, the c-Met antibody (hu8C4) of the present invention, vectibix, a mixture of the c-Met antibody (hu8C4) of the present invention and vectibix, C-EM1 and LA480 were diluted to reach a final concentration of 100 nM, after which the resulting antibodies were treated and reacted for 18 hours. As a plate to be used as a non-decreasing control group with c-Met and EGFR, a human IgG antibody was treated and reacted for 18 hours. Then, cells of each well were collected by 500 $\mu\ell$ of an enzyme-free cell dissociation buffer (Gibco, #13151), after which cells were separated from the enzyme-free cell dissociation buffer by a centrifugal separator, such that the enzyme-free cell dissociation buffer was removed therefrom. For immunofluorescence staining, a goat-derived c-Met antibody (R&D systems, AF276), a goat-derived EGFR antibody (R&D systems, AF231) or a non-specific goat-derived antibody for measuring an amount of staining were mixed by 2 μg respectively with 200 $\mu\ell$ of a cold PBS containing 2% (v/v) FBS, after which the resulting antibodies were treated into each well, such that the resulting plate was reacted at 4°C for 1 hour. Then, the resulting plate was washed twice with a cold PBS containing 2% (v/v) FBS. ALEXA488 was bound as a secondary antibody, after which 1 $\mu\ell$ of a donkey-derived antibody (Thermo Fisher, A-11055) binding to a goat antibody was diluted with 200 $\mu\ell$ of a cold PBS containing 2% (v/v) FBS, such that the resulting antibody was used. After being reacted with the secondary antibody at 4°C for 1 hour, the resulting cells were washed twice with a cold PBS containing 2% (v/v) FBS, after which the resulting cells were fixed by using 200 $\mu\ell$ of BD Cytofix(BD, #554655). After being washed once with PBS, an ALEXA488 Geo-mean (MFI) value, a degree of fluorescent staining, was measured by using a BD FACS Canto II fluorescence activated cell sorter. An amount of c-Met and EGFR located on a cell membrane was indicated as geo mean fluorescence intensity (MFI) by a following formula. With regard to values obtained after repeatedly performing a test three times, an average and standard deviation thereof are shown in Table 27 and FIGS. 18 and 19.

[400]

[401] c-Met or EGFR surface amount = geo $MFI_{[experimental\ group]}$ - geo $MFI_{[non\text{-}specific\ goat\text{-}derived\ antibody]}$

[402]

[403] [Table 27]

[404] Amount of c-Met and EGFR on the surface of cells measured after treating HCC827 cell line with bispecific antibody (hu8C4 x Vectibix scFv), etc.

Treated antibody	c-Met		EGFR	
	Means (geo MFI)	S.D.	Means (geo MFI)	S.D.
human IgG	5653	1032	11494	3276
hu8C4	3436	892	11593	3448
Vectibix	5653	1309	10326	3256
hu8C4 + Vectibix combined	3551	1047	10111	2932
hu8C4 x Vectibix scFv	1689	321	9930	3305
C-EM1	3665	878	11503	3715
C-LA480	3267	764	11655	4156

[405] As seen in Table 27 above, all the antibodies binding to c-Met decreased c-Met on the surface of cells by 40~70%, while antibodies binding to EGFR showed an in-significant effect of decreasing by 10 - 15%. Further considering an effect of reducing c-Met, hu8C4, combination of hu8C4 + Vectibix, C-EM1 and C-LA480 decreased c-Met on the surface of cells by about 40% or so, while hu8C4 x Vectibix scFv decreased c-Met on the surface of cells by 70%, thus showing a more excellent effect of reducing c-Met on the surface of cells than other antibodies and a combination of antibodies.

[406] Results above show that the bispecific antibody (hu8C4 x Vectibix scFv) of the present invention remarkably decreases an amount of c-Met on the surface of cells.

[407]

[408] **Example 20.** Epitope Mapping

[409] To figure out an epitope of the bispecific antibody (hu8C4 x Vectibix scFv) of the present invention on a human c-Met antigen, its analysis was commissioned to the molecule model design support team of the Osong Medical Innovation Foundation (KBIO, Korea). The analysis was performed by hydrogen-deuterium exchange mass spectrometry (HDX-MS).

[410] c-Met sema domain consists of two α/β chains, thus identifying each coverage for the two chains. Due to a presence of a number of disulfide bonds in a sample, a peptide coverage was optimized by adjusting a quench holding time, a TCEP concentration, a pepsin concentration, etc. Finally, an experiment was performed under quench buffer conditions with 100 mM K.Phosphate, 125 mM TCEP, 0.5 M Guanidine-HCl and pH 2.66.

[411] Antigens and antibodies were prepared at a concentration of 3.3 mg/ml and 65 mg/ml respectively, and 37 pmol of cMET antigens and 36 pmol of antibodies were bound 3 hours before the experiment. A deuterium labeling buffer was reacted for 0, 0.33, 10, 60 and 240 minutes. Labeling was stopped with a quench buffer in accordance with each labeling time and vortexing was performed, after which they were immediately frozen in liquid nitrogen, thus being stored at -80°C before the analysis. The resulting antigens and antibodies were loaded onto a pepsin column and analyzed with a mass spectrometer (MS).

[412] As a result of the analysis, it was identified that the bispecific antibody (hu8C4 x Vectibix scFv) of the present invention binds to a 3-dimensional form of epitopes in 4 regions of Y321 - L329 (SEQ. No. 331), I333 - I341 (SEQ. No. 332), P366 - D372 (SEQ. No. 333), and Q464 - S474 (SEQ. No. 334) of a human c-Met sema domain β chain (Table 28). A labeling was performed on a tertiary structure of a human c-Met antigen (PDB No. 4K3J) by using a PyMOL program, wherein results thereof are shown in FIG. 20.

[413]

[414] [Table 28]

[415] Amino acid sequence of epitope region

Epitope region	Amino acids sequence	SEQ ID NO
Y321~L329	YVSKPGAQL	331
I333~I341	IGASLNDDI	332
P366~D372	PIKYVND	333
Q464~S474	QVVVSRSGPST	334

[416] From the results above, it can be seen that the mouse antibody, humanized antibody, affinity-optimized antibody or antigen binding fragments thereof of the present invention, specifically binding to c-Met, selectively act on c-Met, wherein they show an excellent cancer cell proliferation inhibitory activity as well as a remarkably excellent anticancer activity even by a little amount thereof, thus effectively preventing or treating cancer.

[417]

[418] While specific portions of the present invention have been described in detail above, it is apparent to those skilled in the art that such detailed descriptions are set forth to illustrate exemplary embodiments only, but are not construed to limit the scope of the present invention. Thus, it should be understood that the substantial scope of the present invention is defined by the accompanying claims and equivalents thereto.

Claims

[Claim 1] An antibody or an antigen binding fragment thereof that specifically binds to a hepatocyte growth factor receptor (c-Met).

[Claim 2] The antibody or the antigen binding fragment thereof according to claim 1, wherein the antibody binds to one or more epitope region represented by an amino acid sequence selected from the group consisting of SEQ ID NO: 331, SEQ ID NO: 332, SEQ ID NO: 333 and SEQ ID NO: 334.

[Claim 3] The antibody or the antigen binding fragment thereof according to claim 2, wherein the antibody or the antigen binding fragment thereof binds to a human c-Met by K_D of 1 X 10^{-7} M or less, wherein the K_D is measured by surface plasmon resonance (Biacore) analysis.

[Claim 4] The antibody or the antigen binding fragment thereof according to claim 1, wherein the antibody is:

(a) an antibody comprising a light chain variable region comprising a light chain CDR1 represented by SEQ ID NO: 1; a light chain CDR2 represented by SEQ ID NO: 2; a light chain CDR3 represented by SEQ ID NO: 3, and a heavy chain variable region comprising a heavy chain CDR1 represented by SEQ ID NO: 7; a heavy chain CDR2 represented by SEQ ID NO: 8; and a heavy chain CDR3 represented by SEQ ID NO: 9;

(b) an antibody comprising a light chain variable region comprising a light chain CDR1 represented by SEQ ID NO: 4; a light chain CDR2 represented by SEQ ID NO: 5; a light chain CDR3 represented by SEQ ID NO: 6, and a heavy chain variable region comprising a heavy chain CDR1 represented by SEQ ID NO: 10; a heavy chain CDR2 represented by SEQ ID NO: 11; a heavy chain CDR3 represented by SEQ ID NO: 12; or

(c) an affinity-optimized antibodies thereof.

[Claim 5] The antibody or the antigen binding fragment thereof according to claim 4, wherein the antibody comprises: (a) a light chain variable region represented by SEQ ID NO: 13 and a heavy chain variable region represented by SEQ ID NO: 15; or (b) a light chain variable region represented by SEQ ID NO: 14 and a heavy chain variable region represented by SEQ ID NO: 16.

[Claim 6] The antibody or the antigen binding fragment thereof according to claim 4, wherein the antibody comprises:

(a) a light chain variable region represented by SEQ ID NO: 21 and a heavy chain variable region represented by SEQ ID NO: 23;
(b) a light chain variable region represented by SEQ ID NO: 22 and a heavy chain variable region represented by SEQ ID NO: 24;
(c) a light chain variable region represented by SEQ ID NO: 29 and a heavy chain variable region represented by SEQ ID NO: 31; or
(d) a light chain variable region represented by SEQ ID NO: 30 and a heavy chain variable region represented by SEQ ID NO: 32.

[Claim 7] The antibody or the antigen binding fragment thereof according to claim 4, wherein the antibody comprises a hinge region represented by any one of SEQ ID NO: 37 to SEQ ID NO: 44.

[Claim 8] The antibody or the antigen binding fragment thereof according to claim 4, wherein the affinity-optimized antibody is an antibody, in which at least one amino acid sequence is substituted from an antibody, comprising:
a light chain variable region comprising a light chain CDR1 represented by SEQ ID No: 1; a light chain CDR2 represented by SEQ ID No: 2; a light chain CDR3 represented by SEQ ID No: 3, and a heavy chain variable region comprising a heavy chain CDR1 represented by SEQ ID No: 7; a heavy chain CDR2 represented by SEQ ID No: 8; and a heavy chain CDR3 represented by SEQ ID No: 9; and wherein
(i) G in a 1st position of the light chain CDR1 is substituted with A, E, K, L, N, R, S, V or W; A in a 2nd position thereof is substituted with C, G, I, P, S, T or V; S in a 3rd position thereof is substituted with G, M, N, P, Q, R, S or T; E in a 4th position thereof is substituted with A, D, F, G, H, K, M, Q, R, S, T or V; N in a 5th position thereof is substituted with A, D, E, G, K, L, P, Q, R, S, T or V; I in a 6th position thereof is substituted with A, F, L, M, Q, R, S, T or V; Y in a 7th position thereof is substituted with F, H, R or V; or G in a 8th position thereof is substituted with D, F, H, M, N, R, S, T or V;
(ii) G in a 1st position of the light chain CDR2 is substituted with D, F, H, K, P, Q, S, V or Y; T in a 3rd position thereof is substituted with Q; or N in a 4th position thereof is substituted with G;
(iii) Q in a 1st position of the light chain CDR3 is substituted with E, G, I, M or N; N in a 2nd position thereof is substituted with A, D, E, H, L, Q, S or T; V in a 3rd position thereof is substituted with I, L, M, N, Q, S or T; L in a 4th position thereof is substituted with F, H, I, M, R, S, V, W or Y; S in a 5th position thereof is substituted with C, D, E, F, G,

H, K, L, N, Q, R, T, V or Y; S in a 6th position thereof is substituted with D, E, F, G, H, I, L, M, N, P, Q, R, T, V or Y; P in a 7th position thereof is substituted with A, D, E, G, N, Q, S or V; Y in an 8th position thereof is substituted with E, F, L, M or Q; or T in a 9th position thereof is substituted with D, F, G, I, L, N, S, V, W or Y;
(iv) D in a 1st position of the heavy chain CDR1 is substituted with G or Q; Y in a 2nd position thereof is substituted with Q; or I in a 4th position thereof is substituted with A or Q;
(v) F in a 3rd position of the heavy chain CDR2 is substituted with D, E, W or Y; G in a 5th position thereof is substituted with D, H or Y; S in a 6th position thereof is substituted with F, P, W or Y; G in a 7th position thereof is substituted with A, F, L, N or T; N in an 8th position thereof is substituted with F, P, S, T or Y; T in a 9th position thereof is substituted with A, D, E, F, G, H, L, P, S or V; H in a 10th position thereof is substituted with A, D, F, M, R, S, T, V, W or Y; F in an 11th position thereof is substituted with G, H, I, L, M, N, P, Q, V or Y; S in a 12th position thereof is substituted with A, D, G, H, I, L, P, T or V; A in a 13th position thereof is substituted with D, E, F, G, H, I, K, L, M, P, R, S, T, V or Y; R in a 14th position thereof is substituted with A, E, G, H, L, N, P, Q, S, W or Y; F in a 15th position thereof is substituted with D, E, G, L, M, P, R, S, V or W; K in a 16th position thereof is substituted with A, E, F, G, H, L, R, S, T, V or Y; or G in a 17th position thereof is substituted with E, F, H, L, M, N, P, Q, R, S, T, V or W; or
(vi) G in a 1st position of the heavy chain CDR3 is substituted with E, F, H, N, Q, V or W; D in a 2nd position thereof is substituted with E; Y in a 3rd position thereof is substituted with L, Q, T or V; G in a 4th position thereof is substituted with W; F in a 5th position thereof is substituted with L or Y; L in a 6th position thereof is substituted with Q, S or Y; or Y in a 7th position thereof is substituted with C, L, M, N or Q,
wherein the light chain CDR1 comprises 0 to 5 substitutions, the light chain CDR2 comprises 0 to 1 substitution, the light chain CDR3 comprises 0 to 7 substitutions, the heavy chain CDR1 comprises 0 to 1 substitution, the heavy chain CDR2 comprises 0 to 11 substitutions, and the heavy chain CDR3 comprises 0 to 6 substitutions.

[Claim 9] The antibody or the antigen binding fragment thereof according to claim 4, wherein the affinity-optimized antibody comprises:
a light chain variable region comprising a light chain CDR1 represented by any one of SEQ ID NO: 1 and SEQ ID NO: 229 to SEQ ID

NO: 268; a light chain CDR2 represented by any one of SEQ ID NO: 2, SEQ ID NO: 182 to SEQ ID NO: 190, SEQ ID NO: 227 and SEQ ID NO: 228; a light chain CDR3 represented by any one of SEQ ID NO: 3, SEQ ID NO: 142 to SEQ ID NO: 181, SEQ ID NO: 191 to SEQ ID NO: 226 and SEQ ID NO: 269 to SEQ ID NO: 301; and
a heavy chain variable region comprising a heavy chain CDR1 represented by any one of SEQ ID NO: 7 and SEQ ID NO: 108 to SEQ ID NO: 112; a heavy chain CDR2 represented by any one of SEQ ID NO: 8, SEQ ID NO: 54 to SEQ ID NO: 63, SEQ ID NO: 72 to SEQ ID NO: 107 and SEQ ID NO: 118 to SEQ ID NO: 141; a heavy chain CDR3 represented by any one of SEQ ID NO: 9, SEQ ID NO: 64 to SEQ ID NO: 71 and SEQ ID NO: 113 to SEQ ID NO: 117.

[Claim 10] The antibody or the antigen binding fragment thereof according to claim 9, wherein the affinity-optimized antibody comprises a light chain variable region represented by any one of SEQ ID NO: 21 and SEQ ID NO: 306 to SEQ ID NO: 311, and a heavy chain variable region represented by any one of SEQ ID NO: 23 and SEQ ID NO: 302 to SEQ ID NO: 305.

[Claim 11] The antibody or the antigen binding fragment thereof according to claim 10, wherein the affinity-optimized antibody comprises:
(a) a light chain variable region represented by SEQ ID NO: 21 and a heavy chain variable region represented by SEQ ID NO: 302;
(b) a light chain variable region represented by SEQ ID NO: 21 and a heavy chain variable region represented by SEQ ID NO: 305;
(c) a light chain variable region represented by SEQ ID NO: 310 and a heavy chain variable region represented by SEQ ID NO: 23;
(d) a light chain variable region represented by SEQ ID NO: 308 and a heavy chain variable region represented by SEQ ID NO: 305;
(e) a light chain variable region represented by SEQ ID NO: 306 and a heavy chain variable region represented by SEQ ID NO: 303;
(f) a light chain variable region represented by SEQ ID NO: 307 and a heavy chain variable region represented by SEQ ID NO: 304;
(g) a light chain variable region represented by SEQ ID NO: 308 and a heavy chain variable region represented by SEQ ID NO: 304;
(h) a light chain variable region represented by SEQ ID NO: 309 and a heavy chain variable region represented by SEQ ID NO: 304;
(i) a light chain variable region represented by SEQ ID NO: 311 and a heavy chain variable region represented by SEQ ID NO: 304; or

(j) a light chain variable region represented by SEQ ID NO: 306 and a heavy chain variable region represented by SEQ ID NO: 302.

[Claim 12] The antibody or the antigen binding fragment thereof according to claim 4, wherein the antibody further specifically binds to an epidermal growth factor receptor (EGFR).

[Claim 13] The antibody or the antigen binding fragment thereof according to claim 12, wherein the antibody is that an antibody or an antigen binding fragment thereof binding to EGFR is linked to one light chain or heavy chain terminus of c-Met specific antibody.

[Claim 14] The antibody or the antigen binding fragment thereof according to claim 12, wherein the antigen binding fragment binding to the EGFR is Fab, Fab', $F(ab')_2$ or Fv.

[Claim 15] The antibody or the antigen binding fragment thereof according to claim 12, wherein the antigen binding fragment binding to the EGFR is Fab, Fab', $F(ab')_2$ or Fv.

[Claim 16] The antibody or the antigen binding fragment thereof according to claim 15, wherein the Erbitux scFv comprises an amino acid sequence represented by SEQ ID NO: 313 or SEQ ID NO: 314.

[Claim 17] The antibody or the antigen binding fragment thereof according to claim 15, wherein the Vectibix scFv comprises an amino acid sequence represented by SEQ ID NO: 315.

[Claim 18] The antibody or the antigen binding fragment thereof according to claim 13, wherein the antibody or the antigen binding fragment thereof is linked by a connector represented by SEQ ID NO: 312.

[Claim 19] The antibody or the antigen binding fragment thereof according to claim 4, wherein the antigen binding fragment is Fab, Fab', $F(ab')_2$ or Fv.

[Claim 20] A nucleic acid molecule encoding the antibody or the antigen binding fragment thereof of any one of claims 1 to 19.

[Claim 21] An expression vector comprising the nucleic acid molecule of claim 20.

[Claim 22] A host cell having the expression vector introduced therein of claim 21.

[Claim 23] A method for producing an antibody or an antigen binding fragment thereof, using the host cell of claim 22.

[Claim 24] A composition for detecting c-Met, comprising the antibody or the antigen binding fragment thereof of any one of claims 1 to 19.

[Claim 25] A kit for detecting c-Met, comprising the composition for detecting c-Met of claim 24.

[Claim 26] A method for detecting a c-Met antigen using the antibody or the

antigen binding fragment thereof of any one of claims 1 to 19.

[Claim 27] A composition for preventing or treating cancer, comprising the antibody or the antigen binding fragment of any one of claims 1 to 19.

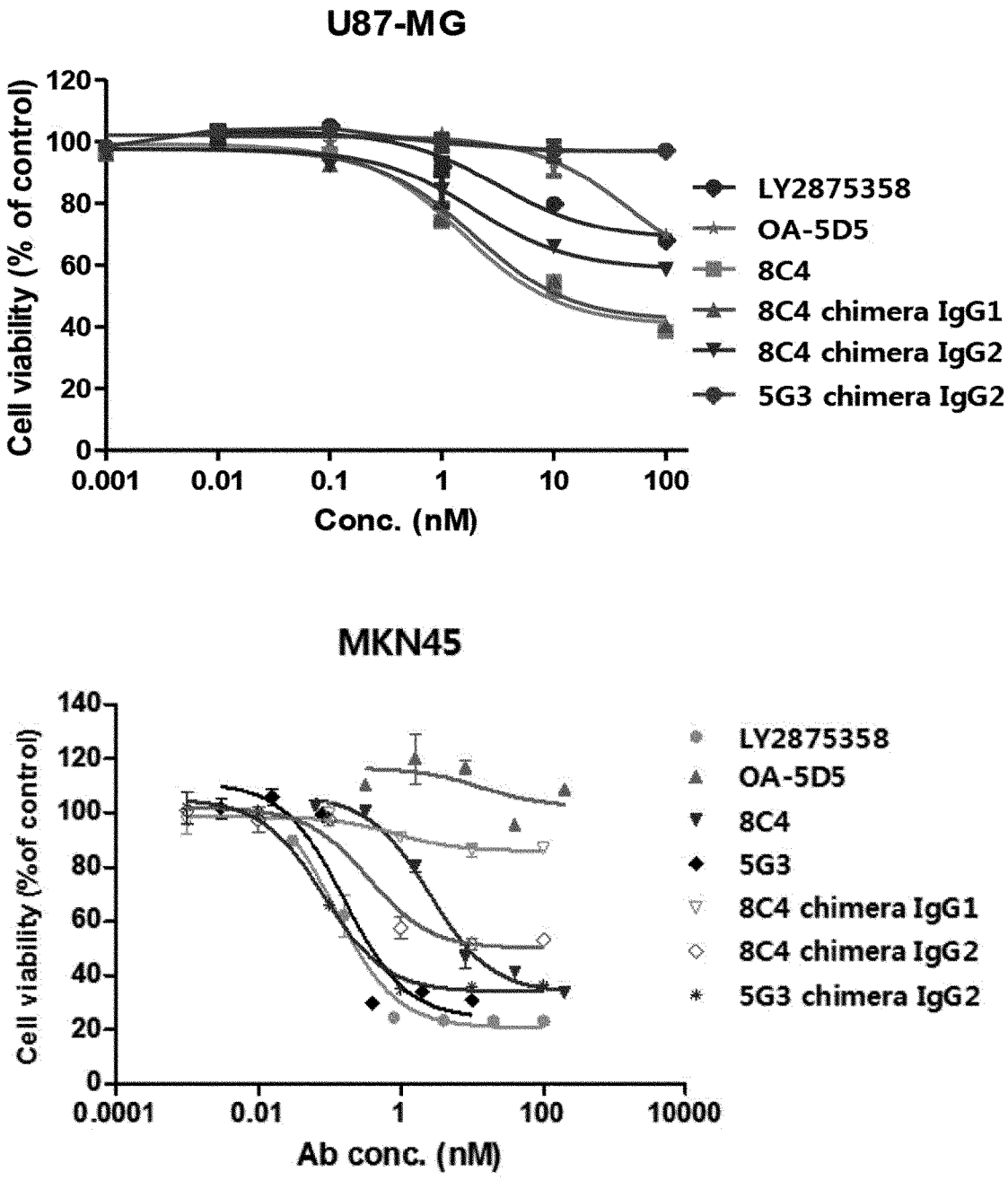
[Claim 28] The composition for preventing or treating cancer according to claims 27, wherein the antibody or the antigen binding fragment thereof binds to c-Met to inhibit a receptor activity.

[Claim 29] The composition for preventing or treating cancer according to claim 28, wherein the antibody or the antigen binding fragment thereof further binds to EGFR to inhibit the receptor activity.

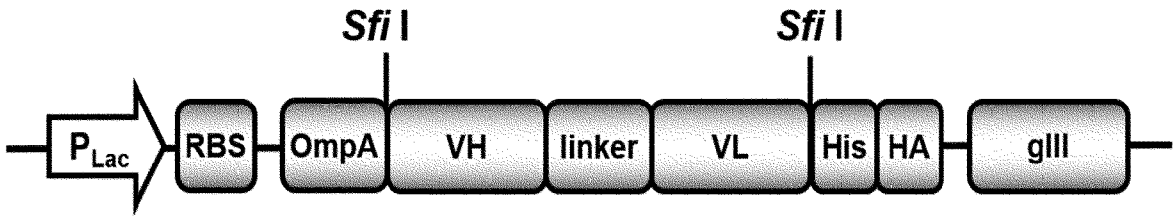
[Claim 30] The composition for preventing or treating cancer according to claim 27, wherein the cancer is caused by c-Met overexpression, amplification, mutation or activation.

[Claim 31] The composition for preventing or treating cancer according to claim 27, wherein the cancer is selected from the group consisting of lung cancer, stomach cancer, colon cancer, rectal cancer, triple negative breast cancer (TNBC), glioblastoma, pancreatic cancer, head and neck cancer, breast cancer, ovarian cancer, liver cancer, renal cancer, bladder cancer, prostate cancer, brain cancer, uterine cancer, solenoma, thyroid cancer, acute myeloid leukemia, chronic myeloid leukemia, myeloma, multiple myeloma, melanoma, lymphoma and adrenal cortex cancer.

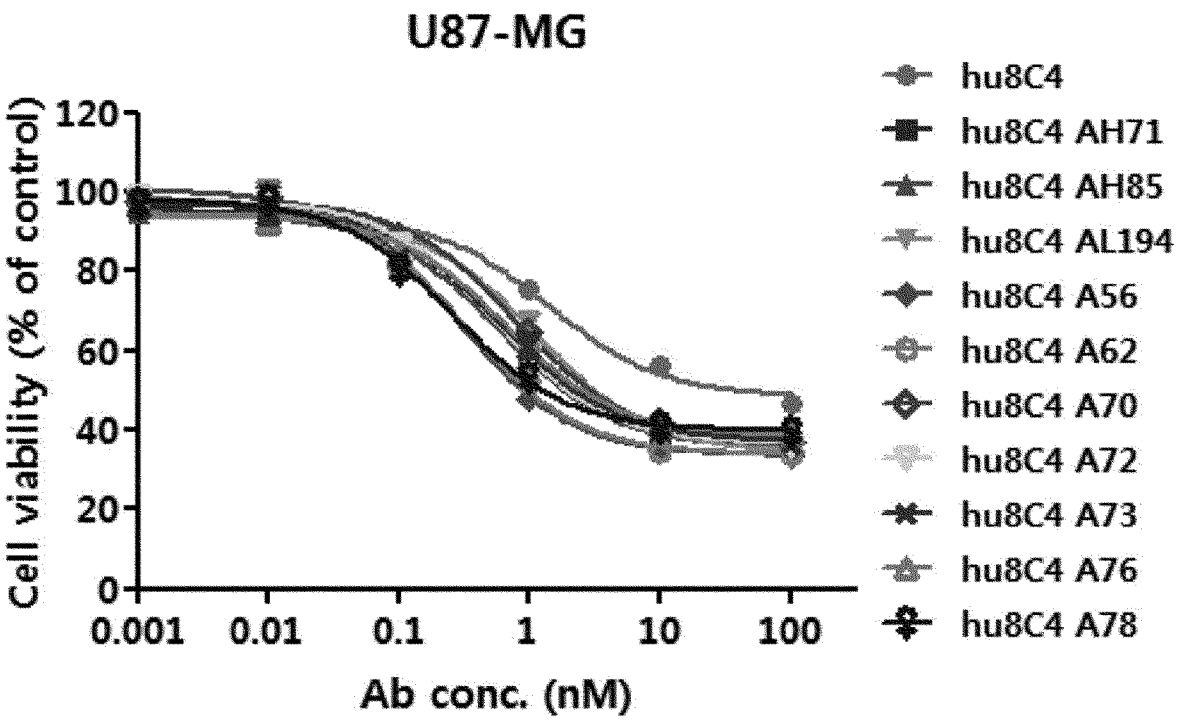
[Fig. 1]


U87-MG
Cell viability (% of control)
120
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
Conc. (nM)
LY2875358
OA-5D5
8C4
8C4 chimera IgG1
8C4 chimera IgG2
5G3 chimera IgG2
MKN45
Cell viability (%of control)
140
120
100
80
60
40
20
0
0.0001
0.01
1
100
10000
Ab conc. (nM)
LY2875358
OA-5D5
8C4
5G3
8C4 chimera IgG1
8C4 chimera IgG2
5G3 chimera IgG2

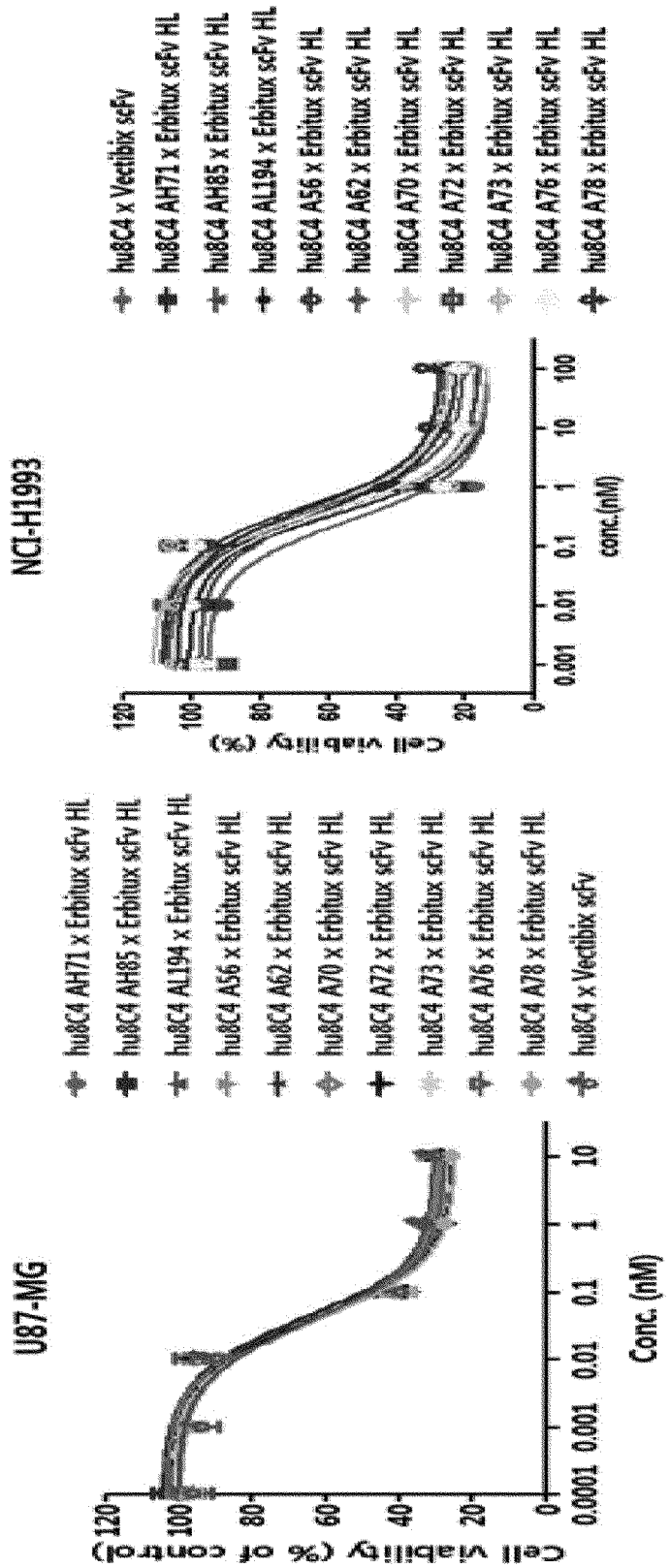
[Fig. 2]


Sfi I
Sfi I
P Lac
RBS
OmpA
VH
linker
VL
His
HA
gIII

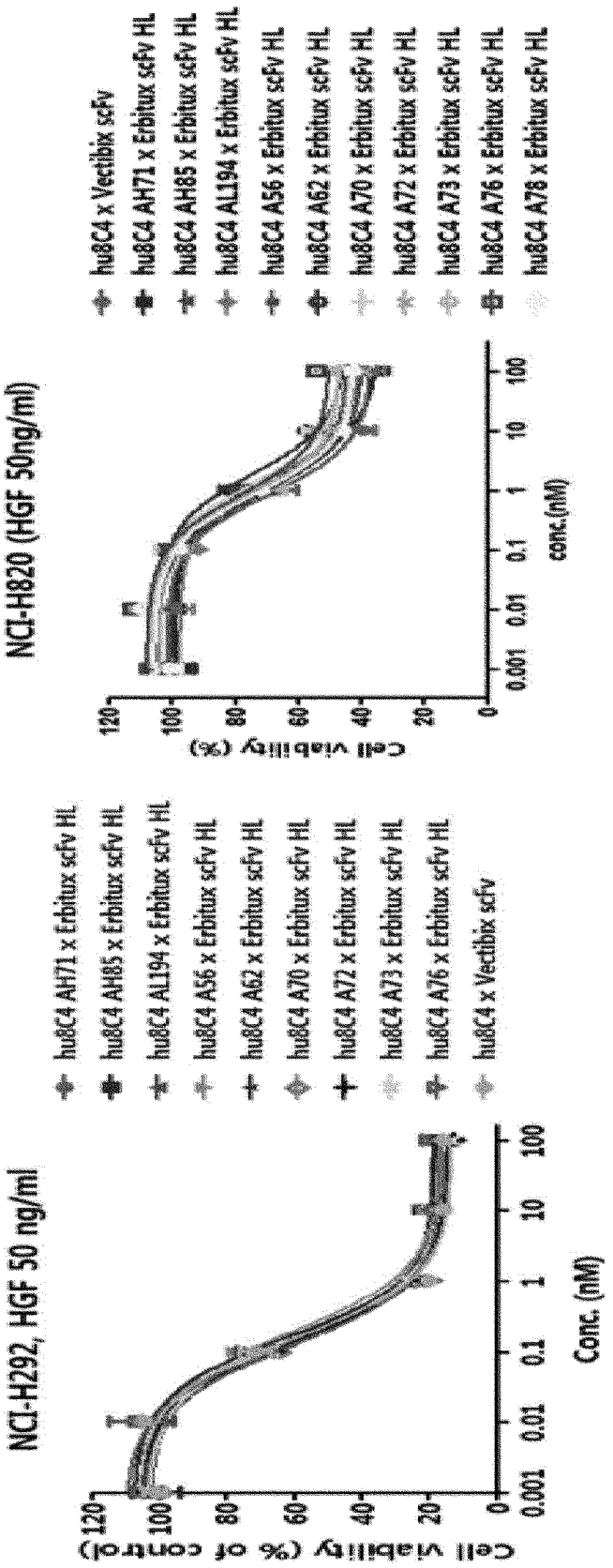
[Fig. 3]


U87-MG
Cell viability (% of control)
120
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
Ab conc. (nM)
hu8C4
hu8C4 AH71
hu8C4 AH85
hu8C4 AL194
hu8C4 A56
hu8C4 A62
hu8C4 A70
hu8C4 A72
hu8C4 A73
hu8C4 A76
hu8C4 A78

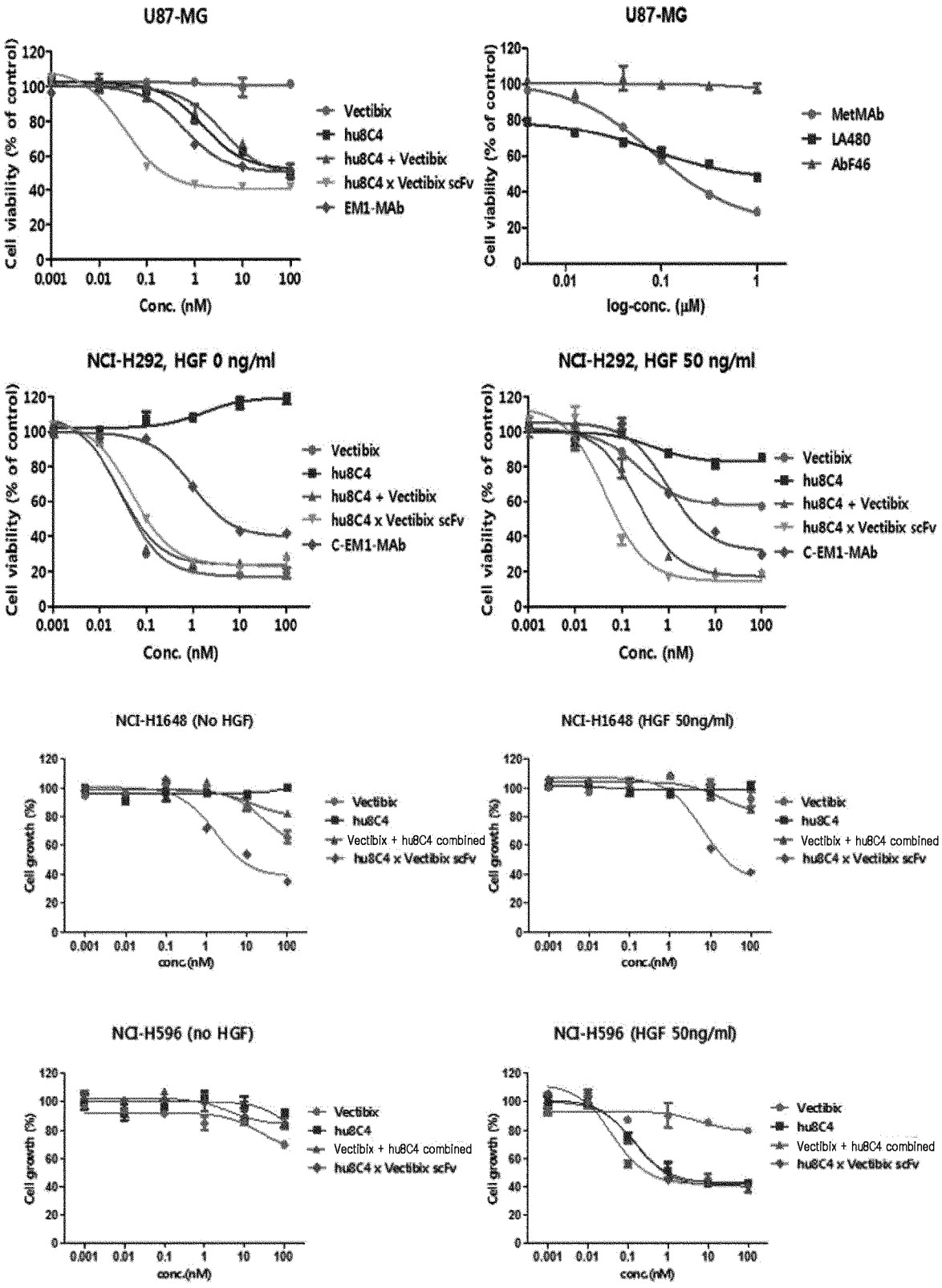
[Fig. 4]


U87-MG
Cell viability (% of control)
120
100
80
60
40
20
0
0.0001
0.001
0.01
0.1
1
10
Conc. (nM)
hu8C4 AH71 x Erbitux scFv HL
hu8C4 AH85 x Erbitux scFv HL
hu8C4 AL194 x Erbitux scFv HL
hu8C4 A56 x Erbitux scFv HL
hu8C4 A62 x Erbitux scFv HL
hu8C4 A70 x Erbitux scFv HL
hu8C4 A72 x Erbitux scFv HL
hu8C4 A73 x Erbitux scFv HL
hu8C4 A76 x Erbitux scFv HL
hu8C4 A78 x Erbitux scFv HL
hu8C4 x Vectibix scFv
NCI-H1993
Cell viability (%)
120
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
conc.(nM)
hu8C4 x Vectibix scFv
hu8C4 AH71 x Erbitux scFv HL
hu8C4 AH85 x Erbitux scFv HL
hu8C4 AL194 x Erbitux scFv HL
hu8C4 A56 x Erbitux scFv HL
hu8C4 A62 x Erbitux scFv HL
hu8C4 A70 x Erbitux scFv HL
hu8C4 A72 x Erbitux scFv HL
hu8C4 A73 x Erbitux scFv HL
hu8C4 A76 x Erbitux scFv HL
hu8C4 A78 x Erbitux scFv HL

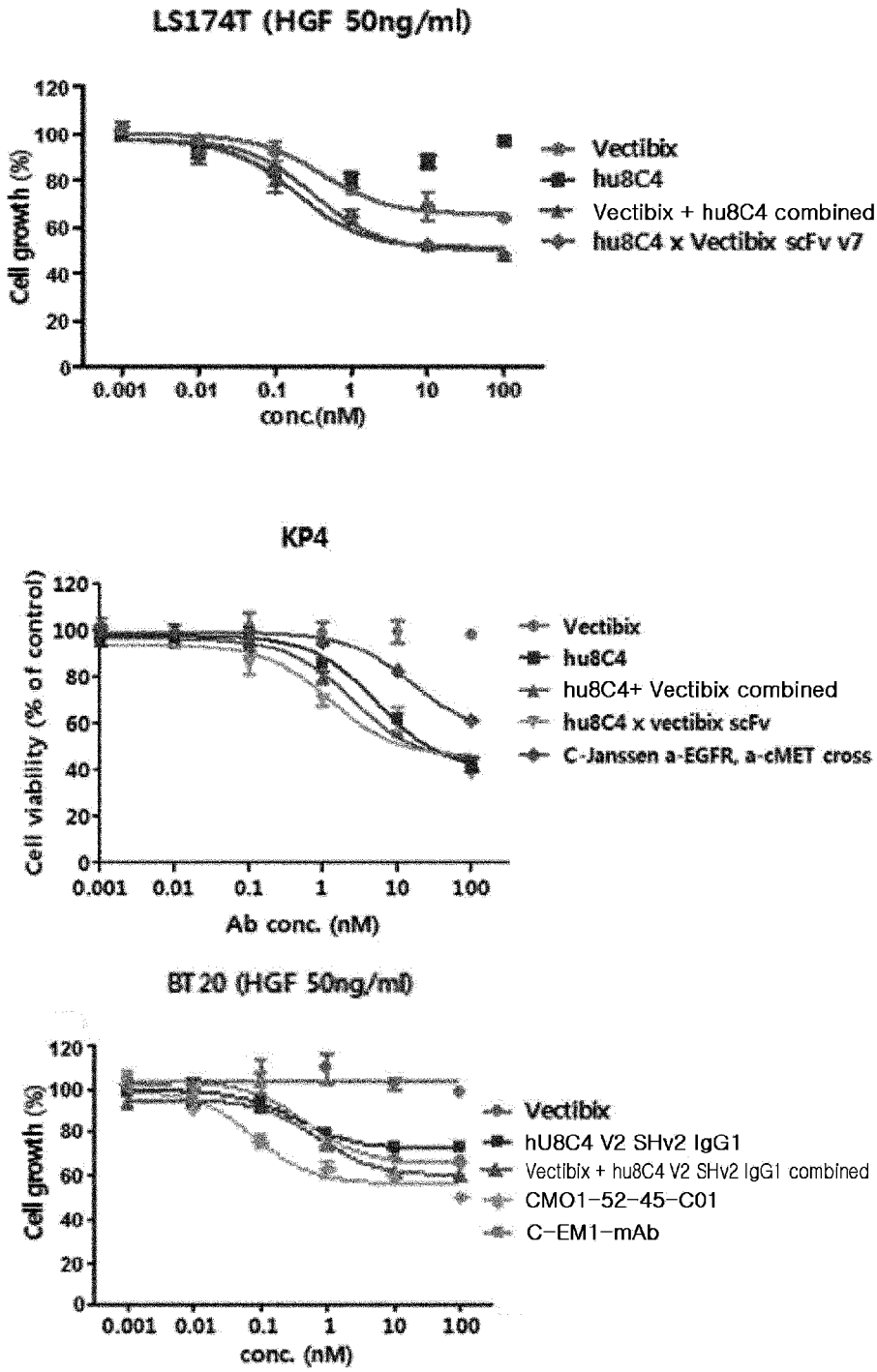
[Fig. 5]


NCI-H820 (HGF 50ng/ml)
Cell viability (%)
conc.(nM)
hu8C4 x Vectibix scFv
hu8C4 AH71 x Erbitux scFv HL
hu8C4 AH85 x Erbitux scFv HL
hu8C4 AL194 x Erbitux scFv HL
hu8C4 A56 x Erbitux scFv HL
hu8C4 A62 x Erbitux scFv HL
hu8C4 A70 x Erbitux scFv HL
hu8C4 A72 x Erbitux scFv HL
hu8C4 A73 x Erbitux scFv HL
hu8C4 A76 x Erbitux scFv HL
hu8C4 A78 x Erbitux scFv HL
NCI-H292, HGF 50 ng/ml
Cell viability (% of control)
Conc. (nM)

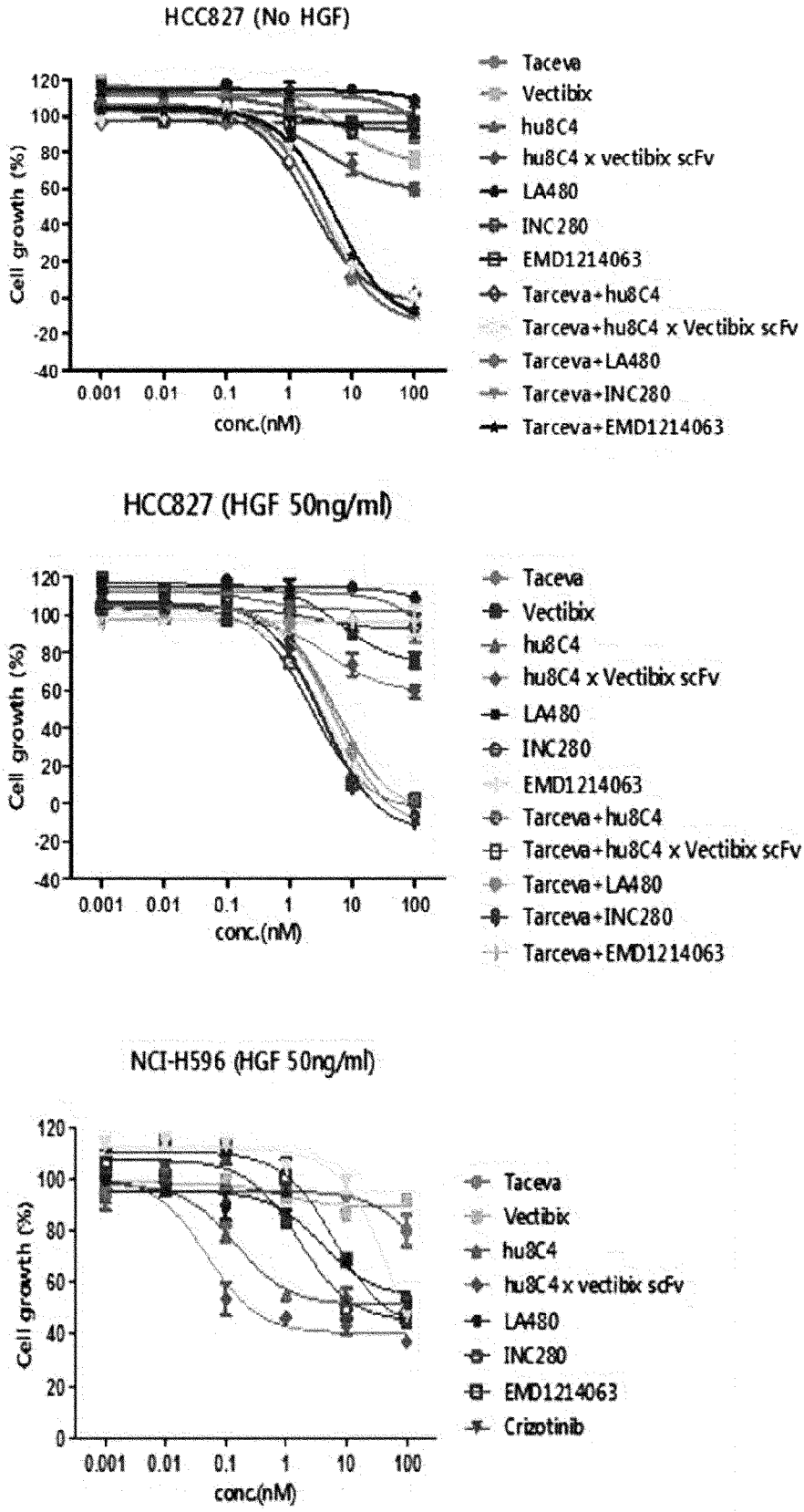
[Fig. 6]


U87-MG
Cell viability (% of control)
Conc. (nM)
Vectibix
hu8C4
hu8C4 + Vectibix
hu8C4 x Vectibix scFv
EM1-MAb
U87-MG
Cell viability (% of control)
log-conc. (μM)
MetMAb
LA480
AbF46
NCI-H292, HGF 0 ng/ml
Cell viability (% of control)
Conc. (nM)
Vectibix
hu8C4
hu8C4 + Vectibix
hu8C4 x Vectibix scFv
C-EM1-MAb
NCI-H292, HGF 50 ng/ml
Cell viability (% of control)
Conc. (nM)
Vectibix
hu8C4
hu8C4 + Vectibix
hu8C4 x Vectibix scFv
C-EM1-MAb
NCI-H1648 (No HGF)
Cell growth (%)
conc.(nM)
Vectibix
hu8C4
Vectibix + hu8C4 combined
hu8C4 x Vectibix scFv
NCI-H1648 (HGF 50ng/ml)
Cell growth (%)
conc.(nM)
Vectibix
hu8C4
Vectibix + hu8C4 combined
hu8C4 x Vectibix scFv
NCI-H596 (no HGF)
Cell growth (%)
conc.(nM)
Vectibix
hu8C4
Vectibix + hu8C4 combined
hu8C4 x Vectibix scFv
NCI-H596 (HGF 50ng/ml)
Cell growth (%)
conc.(nM)
Vectibix
hu8C4
Vectibix + hu8C4 combined
hu8C4 x Vectibix scFv

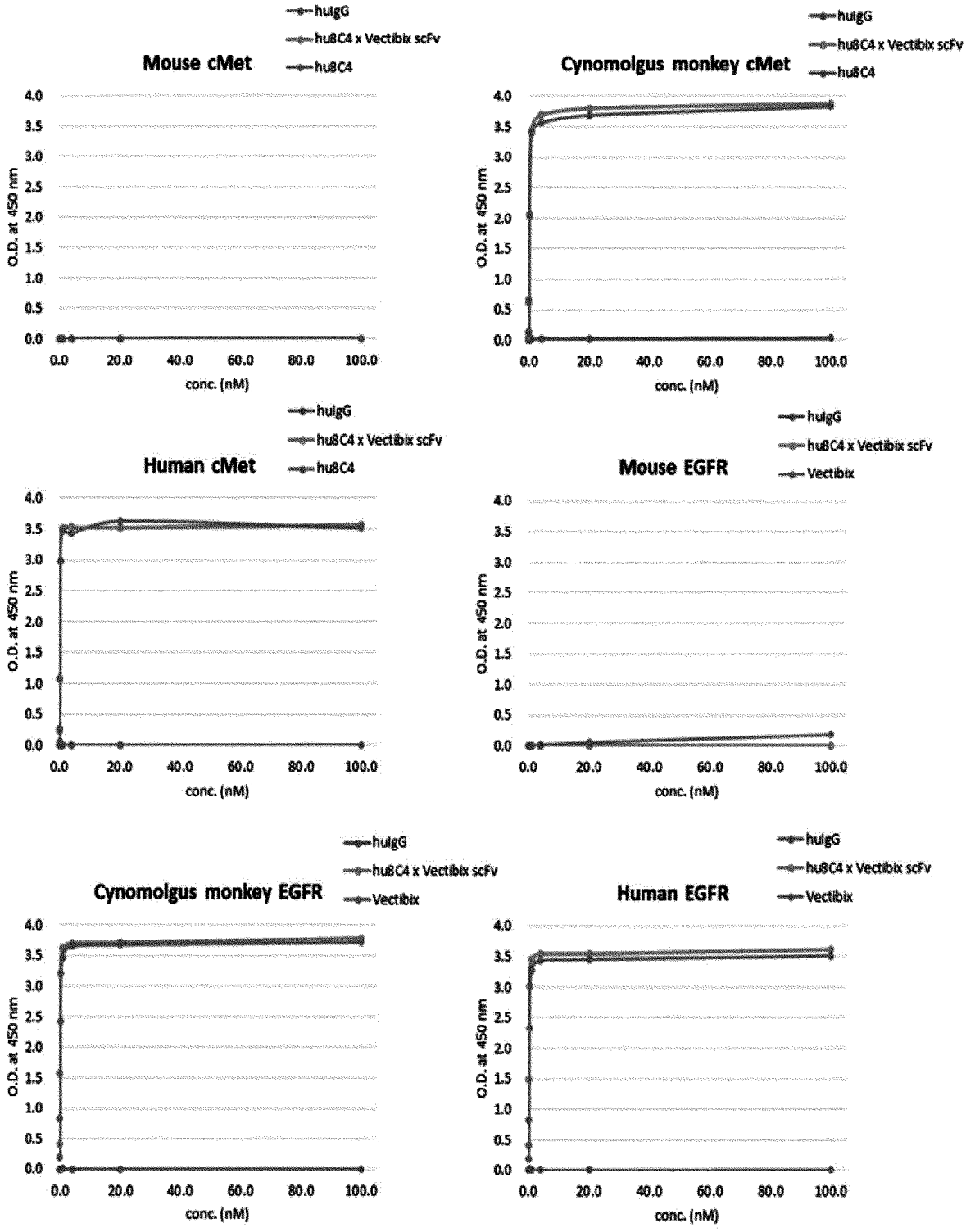
[Fig. 7]


LS174T (HGF 50ng/ml)
Cell growth (%)
conc.(nM)
Vectibix
hu8C4
Vectibix + hu8C4 combined
hu8C4 x Vectibix scFv v7
KP4
Cell viability (% of control)
Ab conc. (nM)
Vectibix
hu8C4
hu8C4+ Vectibix combined
hu8C4 x vectibix scFv
C-Janssen a-EGFR, a-cMET cross
BT 20 (HGF 50ng/ml)
Cell growth (%)
conc. (nM)
Vectibix
hU8C4 V2 SHv2 IgG1
Vectibix + hu8C4 V2 SHv2 IgG1 combined
CMO1-52-45-C01
C-EM1-mAb

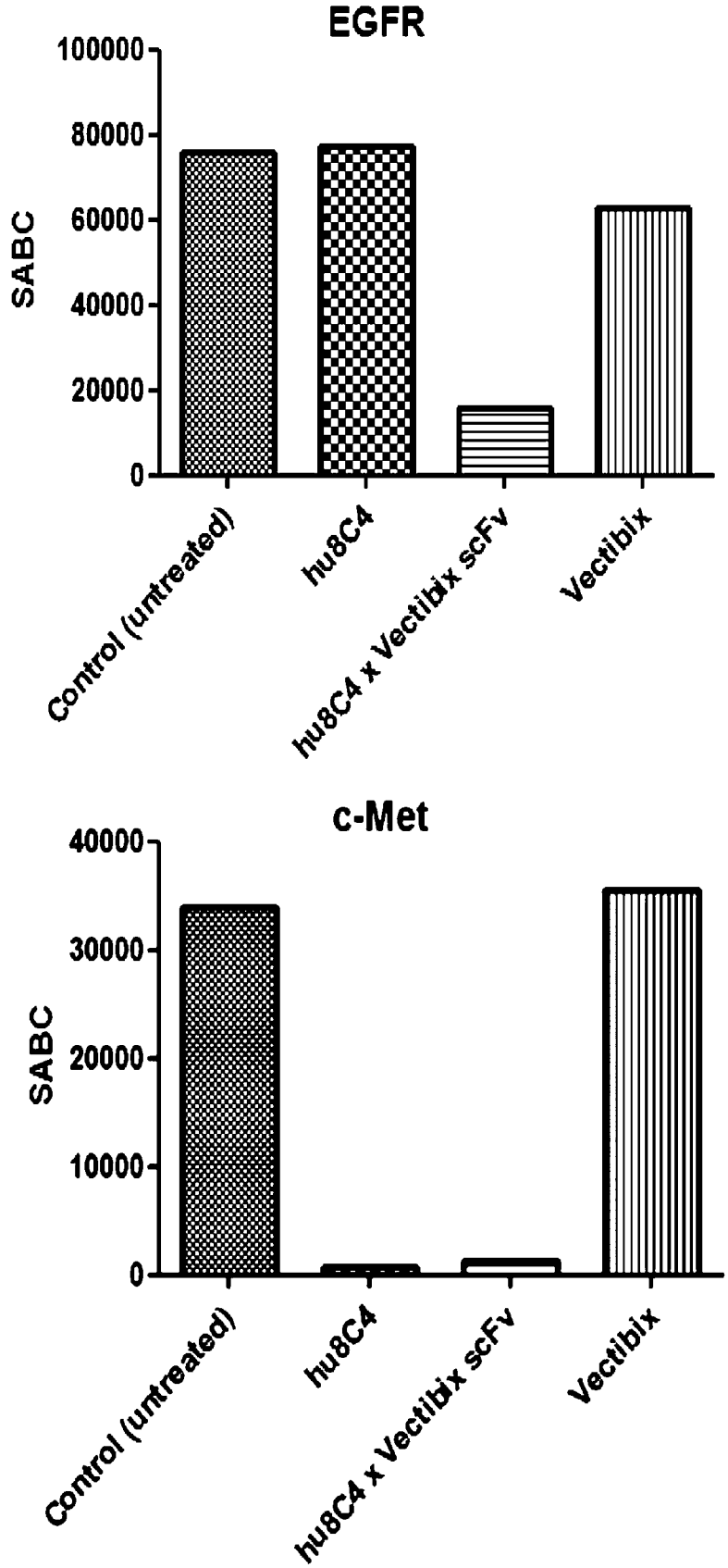
[Fig. 8]


HCC827 (No HGF)
Cell growth (%)
conc.(nM)
Taceva
Vectibix
hu8C4
hu8C4 x vectibix scFv
LA480
INC280
EMD1214063
Tarceva+hu8C4
Tarceva+hu8C4 x Vectibix scFv
Tarceva+LA480
Tarceva+INC280
Tarceva+EMD1214063
HCC827 (HGF 50ng/ml)
Cell growth (%)
conc.(nM)
Taceva
Vectibix
hu8C4
hu8C4 x Vectibix scFv
LA480
INC280
EMD1214063
Tarceva+hu8C4
Tarceva+hu8C4 x Vectibix scFv
Tarceva+LA480
Tarceva+INC280
Tarceva+EMD1214063
NCI-H596 (HGF 50ng/ml)
Cell growth (%)
conc.(nM)
Taceva
Vectibix
hu8C4
hu8C4 x vectibix scFv
LA480
INC280
EMD1214063
Crizotinib

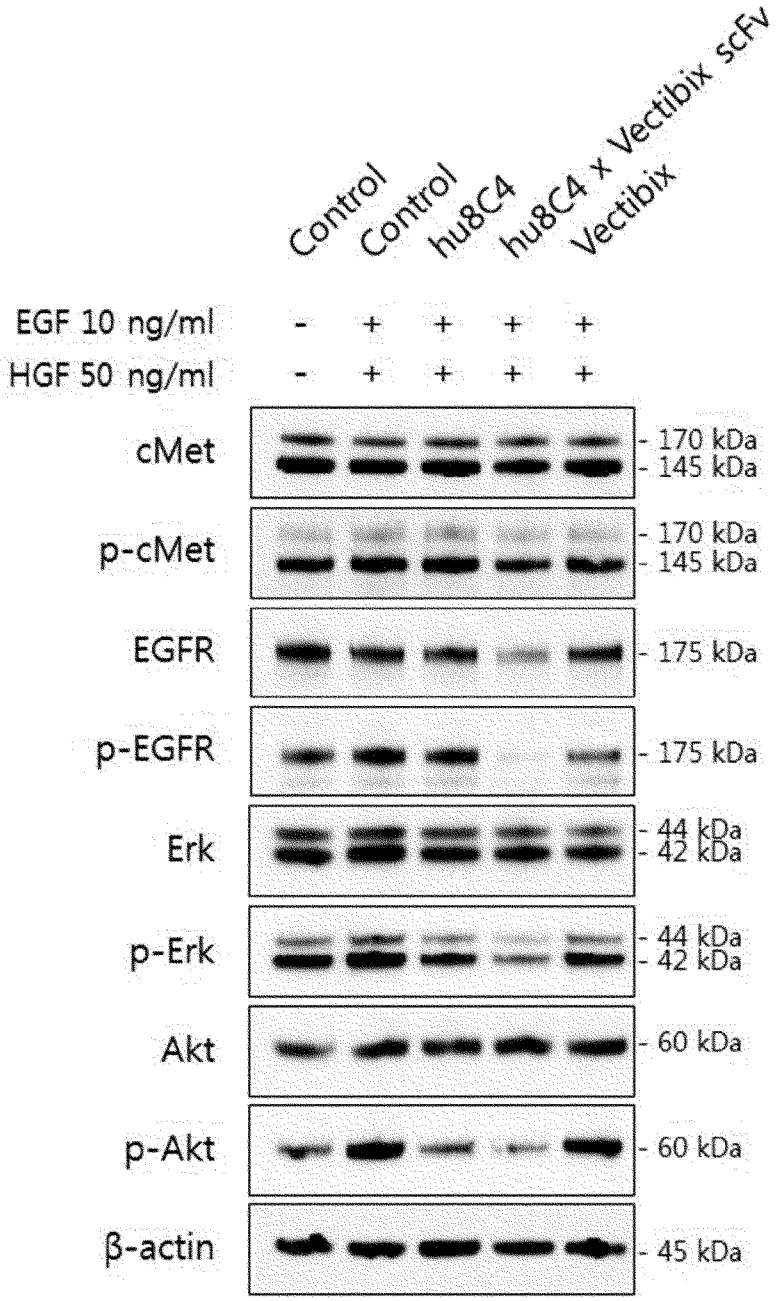
[Fig. 9]


Mouse cMet
huIgG
hu8C4 x Vectibix scFv
hu8C4
O.D. at 450 nm
conc. (nM)
Cynomolgus monkey cMet
Human cMet
Mouse EGFR
Vectibix
Cynomolgus monkey EGFR
Human EGFR

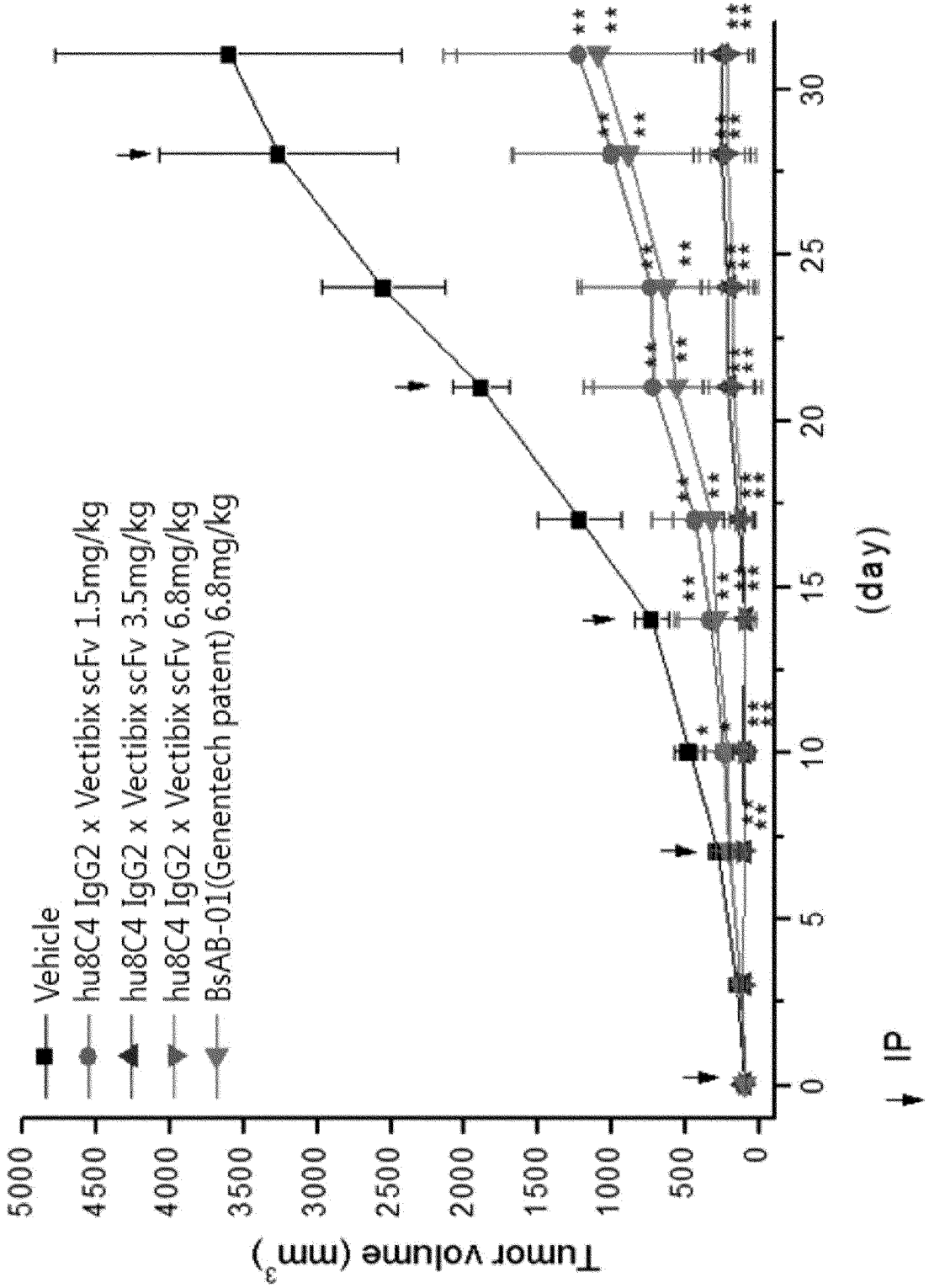
[Fig. 10]


EGFR
SABC
100000
80000
60000
40000
20000
0
Control (untreated)
hu8C4
hu8C4 x Vectibix scFv
Vectibix
c-Met
SABC
40000
30000
20000
10000
0
Control (untreated)
hu8C4
hu8C4 x Vectibix scFv
Vectibix

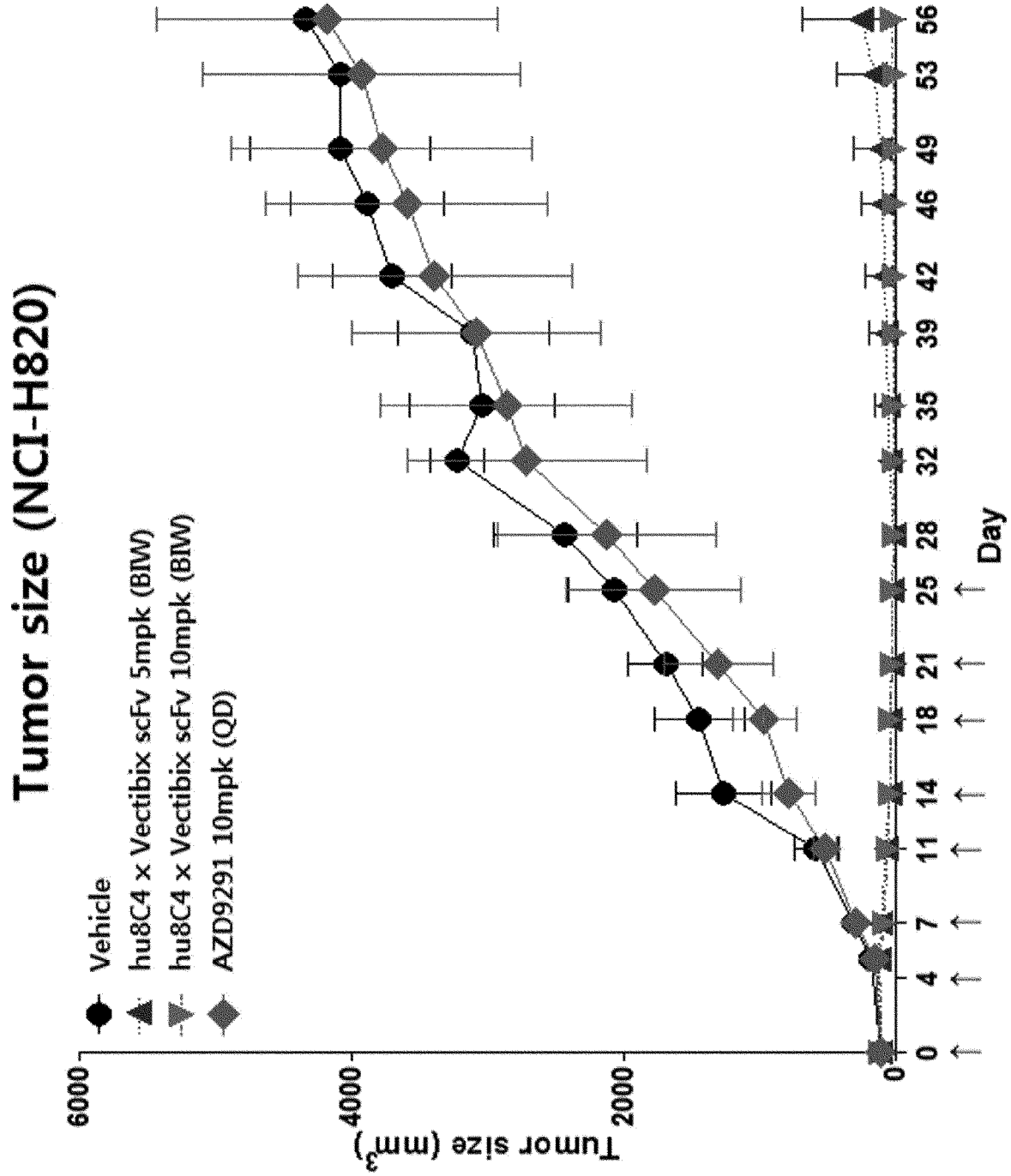
[Fig. 11]


Control
Control
hu8C4
hu8C4 x Vectibix scFv
Vectibix
EGF 10 ng/ml - + + + +
HGF 50 ng/ml - + + + +
cMet
- 170 kDa
- 145 kDa
p-cMet
- 170 kDa
- 145 kDa
EGFR
- 175 kDa
p-EGFR
- 175 kDa
Erk
- 44 kDa
- 42 kDa
p-Erk
- 44 kDa
- 42 kDa
Akt
- 60 kDa
p-Akt
- 60 kDa
β-actin
- 45 kDa

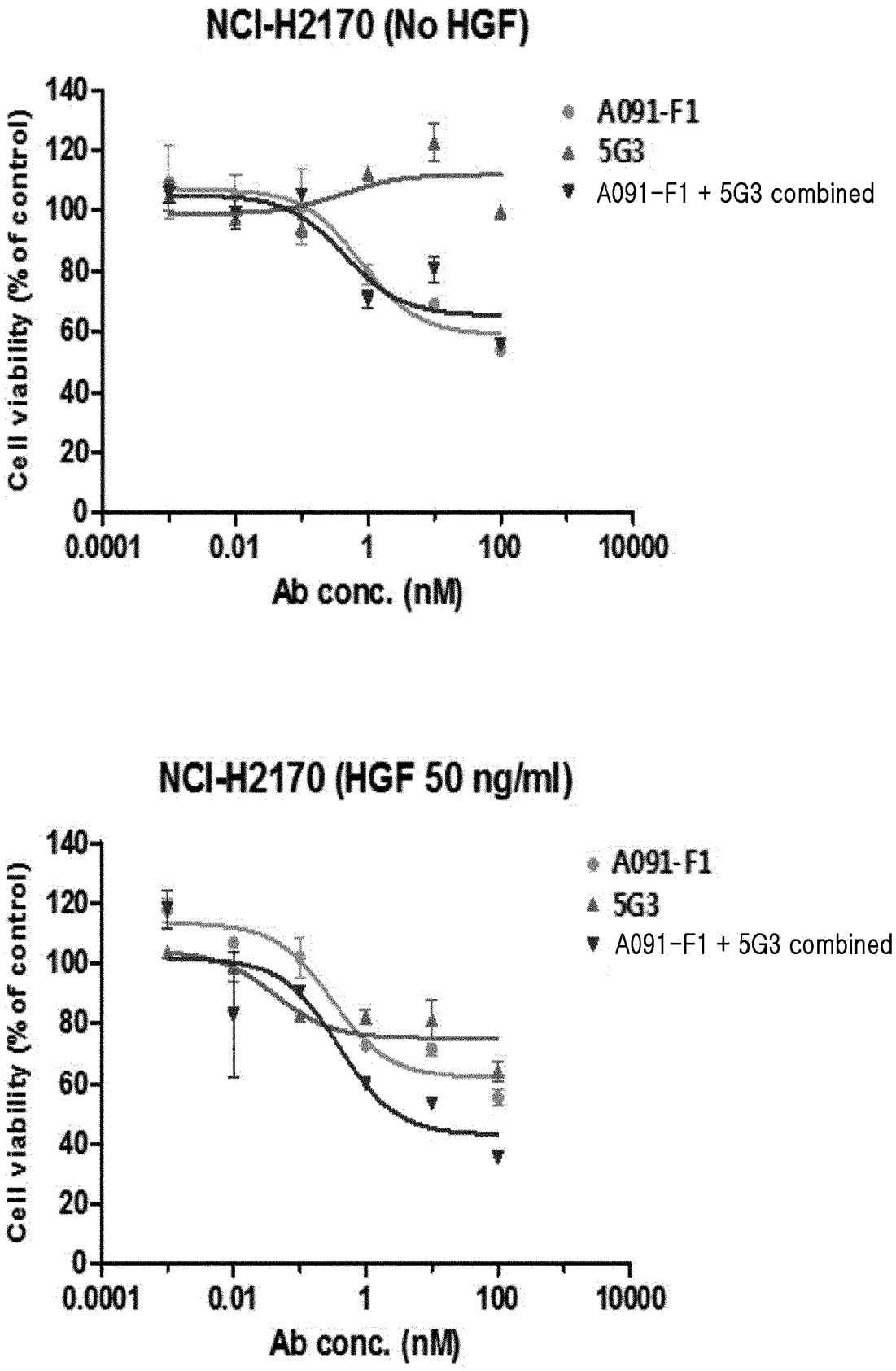
[Fig. 12]


Vehicle
hu8C4 IgG2 x Vectibix scFv 1.5mg/kg
hu8C4 IgG2 x Vectibix scFv 3.5mg/kg
hu8C4 IgG2 x Vectibix scFv 6.8mg/kg
BsAB-01(Genentech patent) 6.8mg/kg
Tumor volume (mm3)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
5
10
15
20
25
30
(day)
IP

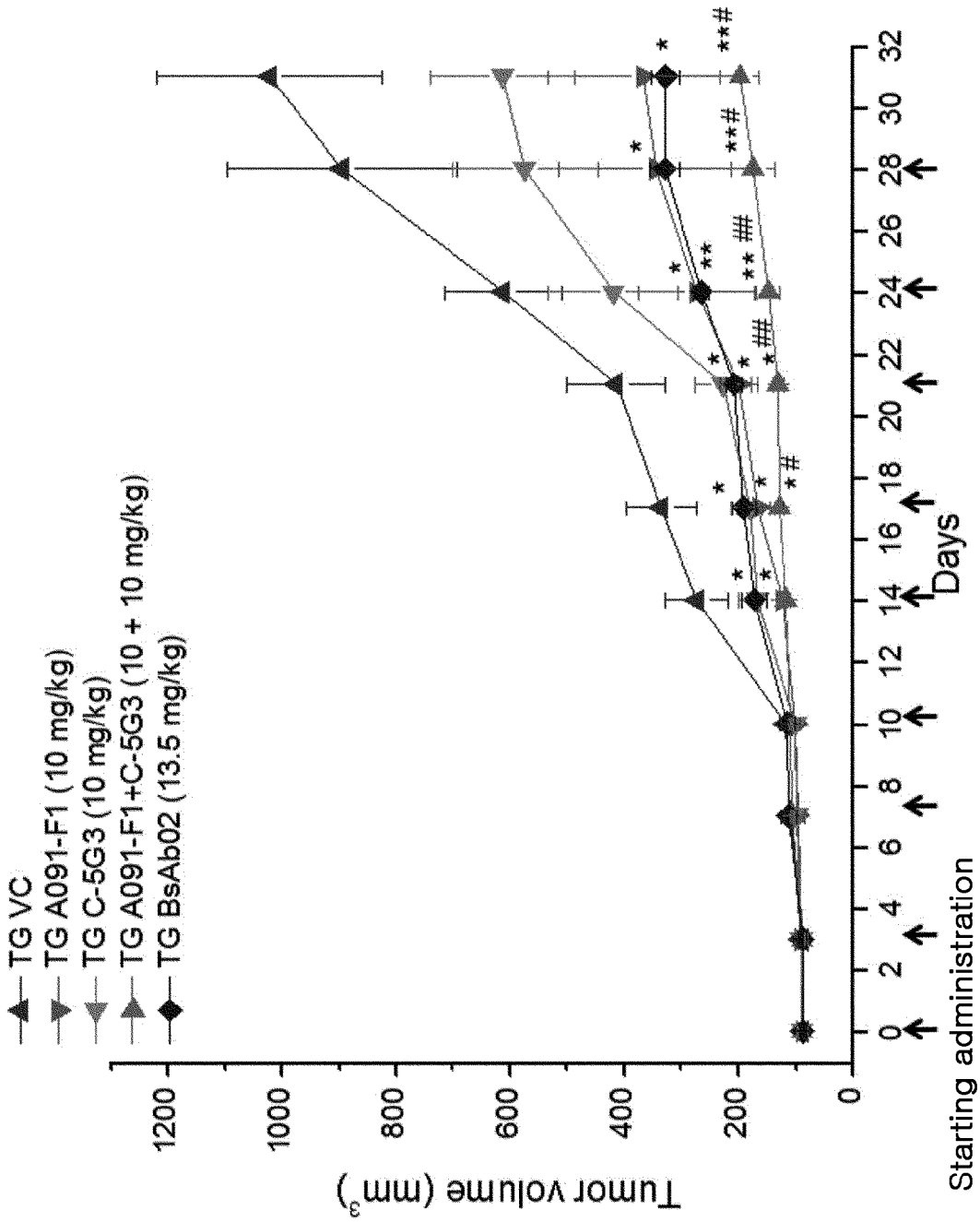
[Fig. 13]


Tumor size (NCI-H820)
Vehicle
hu8C4 x Vectibix scFv 5mpk (BIW)
hu8C4 x Vectibix scFv 10mpk (BIW)
AZD9291 10mpk (QD)
Tumor size (mm3)
6000
4000
2000
0
0 4 7 11 14 18 21 25 28 32 35 39 42 46 49 53 56
Day

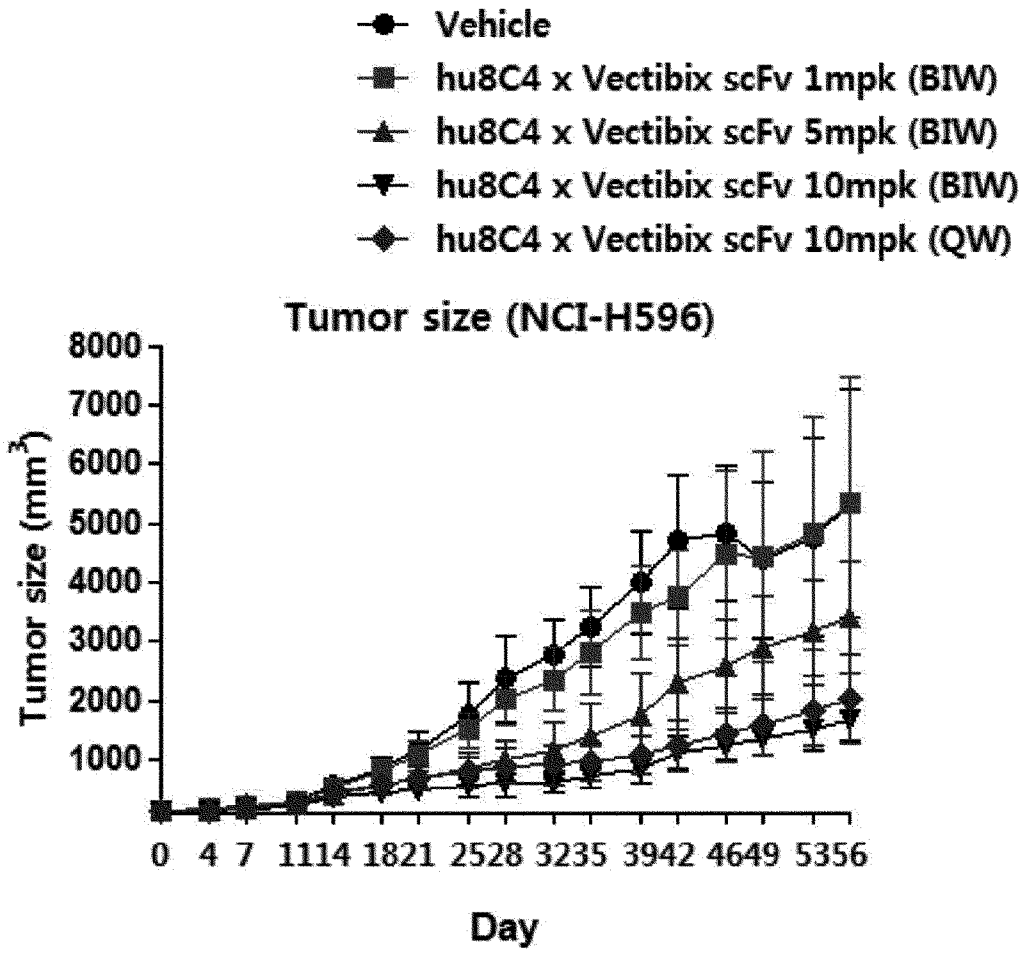
[Fig. 14]


NCI-H2170 (No HGF)
Cell viability (% of control)
140
120
100
80
60
40
20
0
0.0001
0.01
1
100
10000
Ab conc. (nM)
A091-F1
5G3
A091-F1 + 5G3 combined
NCI-H2170 (HGF 50 ng/ml)
Cell viability (% of control)
140
120
100
80
60
40
20
0
0.0001
0.01
1
100
10000
Ab conc. (nM)
A091-F1
5G3
A091-F1 + 5G3 combined

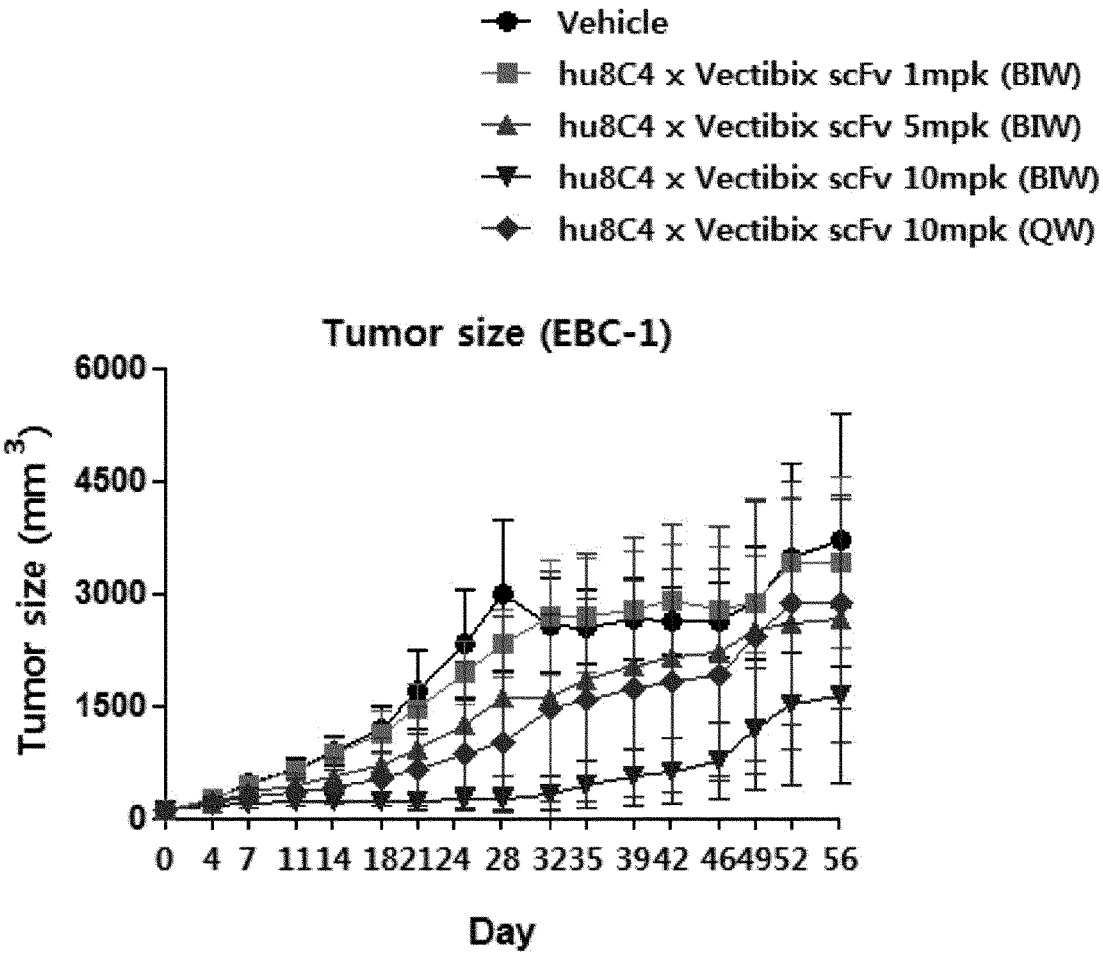
[Fig. 15]


TG VC
TG A091-F1 (10 mg/kg)
TG C-5G3 (10 mg/kg)
TG A091-F1+C-5G3 (10 + 10 mg/kg)
TG BsAb02 (13.5 mg/kg)
Tumor volume (mm³)
0
200
400
600
800
1000
1200
Days
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
Starting administration

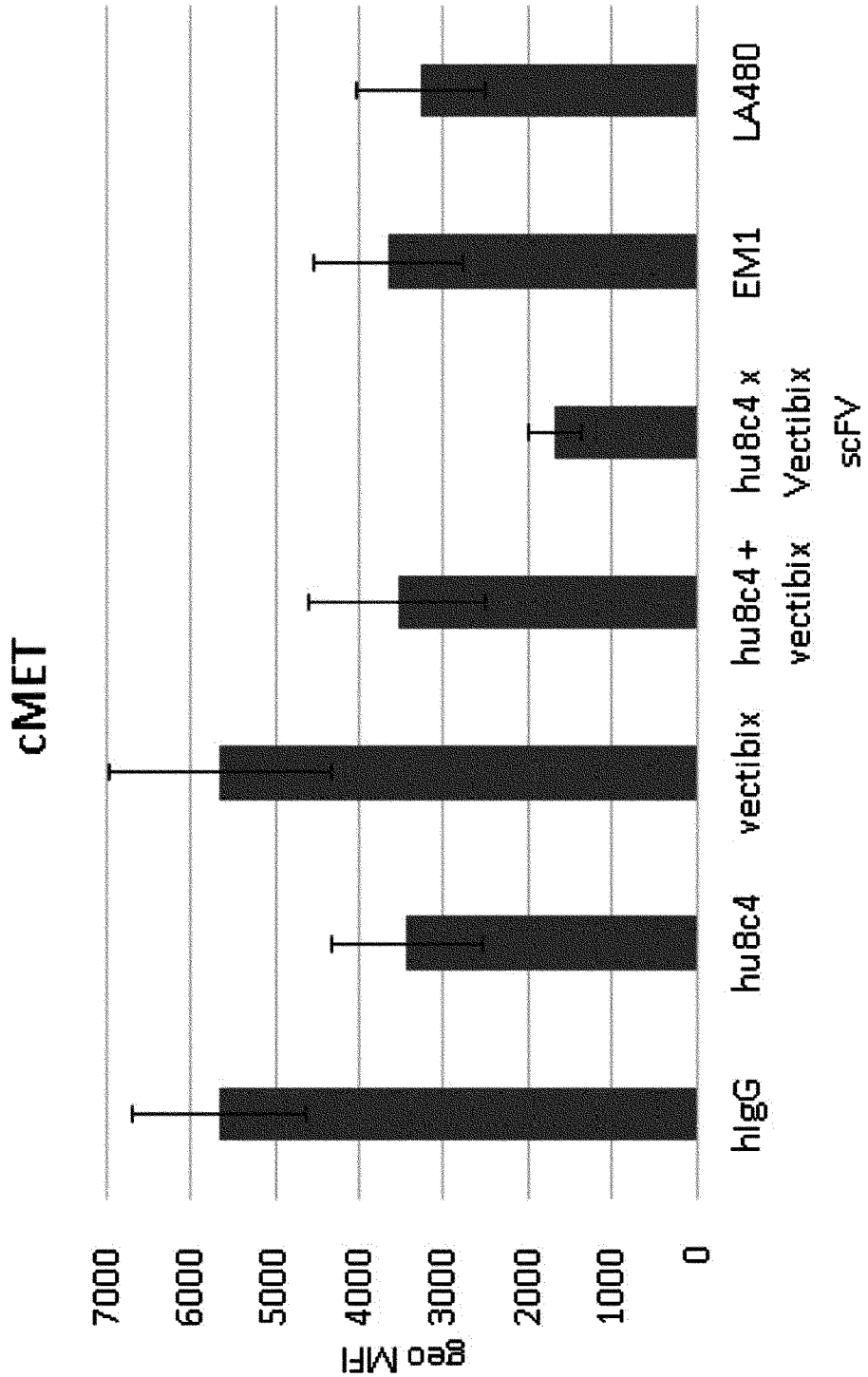
[Fig. 16]


Vehicle
hu8C4 x Vectibix scFv 1mpk (BIW)
hu8C4 x Vectibix scFv 5mpk (BIW)
hu8C4 x Vectibix scFv 10mpk (BIW)
hu8C4 x Vectibix scFv 10mpk (QW)
Tumor size (NCI-H596)
Tumor size (mm3)
8000
7000
6000
5000
4000
3000
2000
1000
0 4 7 1114 1821 2528 3235 3942 4649 5356
Day

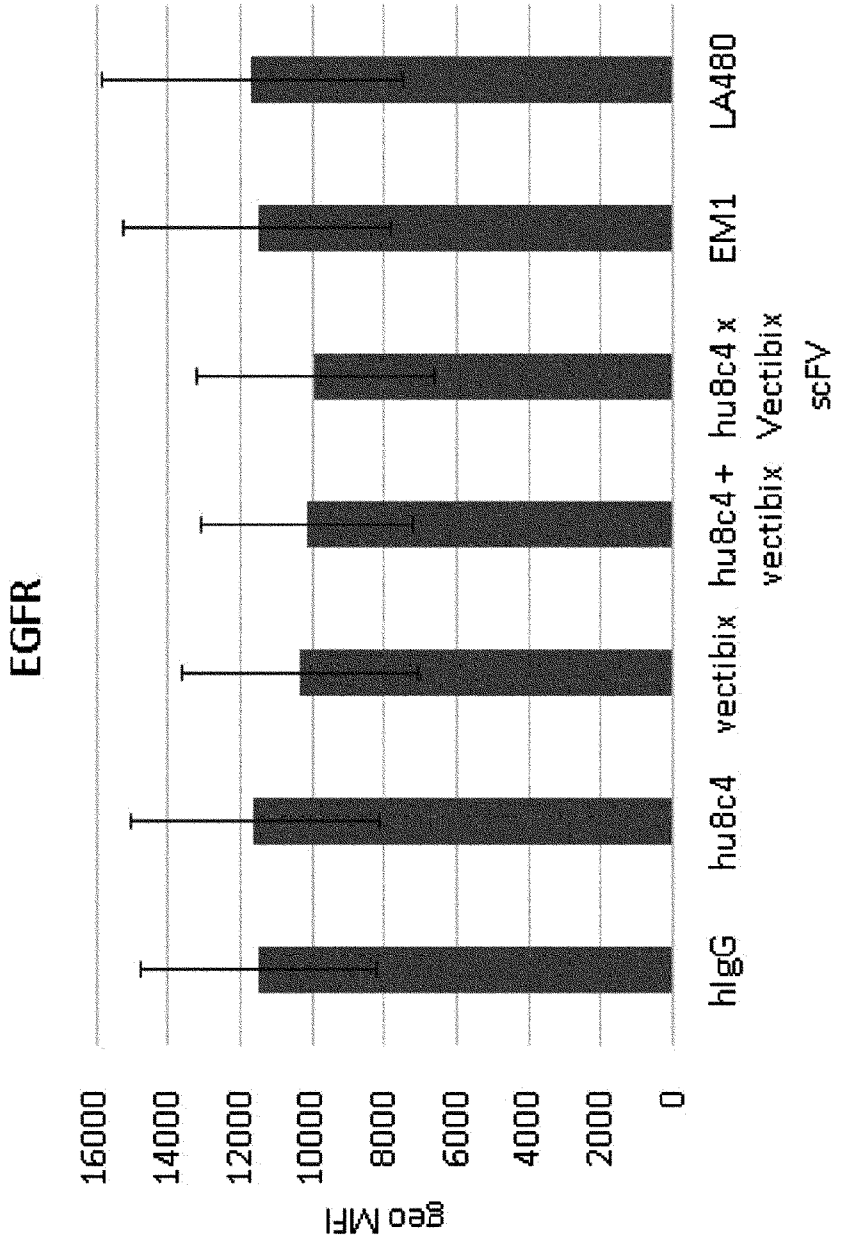
[Fig. 17]


Vehicle
hu8C4 x Vectibix scFv 1mpk (BIW)
hu8C4 x Vectibix scFv 5mpk (BIW)
hu8C4 x Vectibix scFv 10mpk (BIW)
hu8C4 x Vectibix scFv 10mpk (QW)
Tumor size (EBC-1)
Tumor size (mm3)
6000
4500
3000
1500
0
0 4 7 11 14 18 21 24 28 32 35 39 42 46 49 52 56
Day

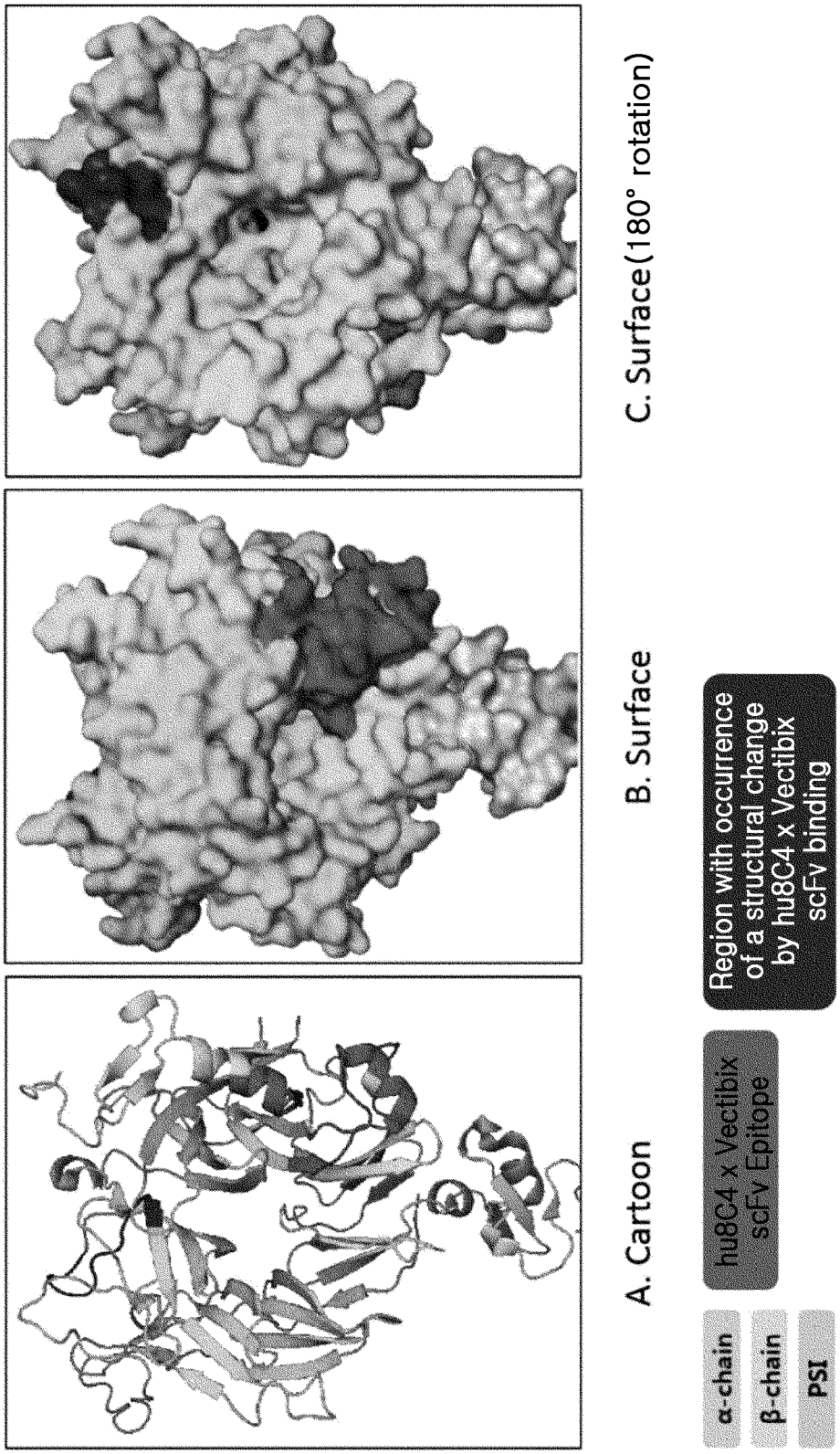
[Fig. 18]


cMET
geo MFI
7000
6000
5000
4000
3000
2000
1000
0
hIgG
hu8c4
vectibix
hu8c4 + vectibix
hu8c4 x Vectibix scFV
EM1
LA480

[Fig. 19]


EGFR
geo MFI
16000
14000
12000
10000
8000
6000
4000
2000
0
hIgG
hu8c4
vectibix
hu8c4 + vectibix
hu8c4 x Vectibix scFV
EM1
LA480

[Fig. 20]


A. Cartoon
B. Surface
C. Surface(180° rotation)
α-chain
β-chain
PSI
hu8C4 x Vectibix scFv Epitope
Region with occurrence of a structural change by hu8C4 x Vectibix scFv binding

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2018/006182

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/28(2006.01)i, G01N 33/574(2006.01)i, A61K 39/00(2006.01)i

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)
C07K 16/28; A61K 39/395; A61K 45/06; A61P 35/00; C07K 16/32; G01N 33/574; A61K 39/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: c-Met antibody, epitope, CDR, substitution, EGFR, bispecific antibody, cancer, treatment

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2014-0141000 A1 (JANSSEN BIOTECH, INC.) 22 May 2014 See claims 1, 2, 19-22, 31, 33-35, 39, 40, 41, 47; and paragraphs [0016], [0057], [0538]-[0545].	1,20-31
A		2-19
X	KR 10-2016-0061199 A (SAMSUNG ELECTRONICS CO., LTD.) 31 May 2016 See abstract; claims 1-3, 12-16; and paragraphs [0203]-[0282].	1,20-31
X	MOORES, SHERI L. et al., `A novel bispecific antibody targeting EGFR and cMet is effective against EGFR inhibitor-resistant lung tumors', Cancer Research, 2016, Vol.76, No.13, pp.3942-3953 See abstract; pages 3943, 3944; and figure 1.	1,20-31
X	US 2015-0118238 A1 (ELI LILLY AND COMPANY) 30 April 2015 See abstract; and claims 2, 15, 17, 28.	1,20-28,30,31
X	US 9631027 B2 (ARGEN-X N.V.) 25 April 2017 See abstract; and claims 1-4.	1,20-28,30,31

☐ 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
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"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	Date of mailing of the international search report
15 November 2018 (15.11.2018)	**15 November 2018 (15.11.2018)**

Name and mailing address of the ISA/KR
International Application Division
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon, 35208, Republic of Korea
Facsimile No. +82-42-481-8578

Authorized officer
KAM, Yoo Lim
Telephone No. +82-42-481-3516

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