

**RECOMBINANT ANTIBODY, PHARMACEUTICAL COMPOSITION COMPRISING
THE SAME, AND USES THEREOF IN TREATING CANCERS**

BACKGROUND OF THE INVENTION

[0001] 1. FIELD OF THE INVENTION

[0002] The present disclosure in general relates to the field of disease treatment. More particularly, the present disclosure relates to recombinant antibodies and their uses in treating cancers.

[0003] 2. DESCRIPTION OF RELATED ART

[0004] Cancer is a disease characterized by the development of abnormal cells that divide uncontrollably and exhibit the potential to infiltrate and destroy normal tissues and/or organs in an affected subject. It is one of the leading causes of death worldwide accounting for nearly 10 million deaths in 2020. The most common cancers include breast cancer, lung cancer, colon and rectum cancer, and prostate cancer. According to the World Health Organization (WHO), about one-third of deaths from cancer are due to tobacco use, high body mass index, alcohol consumption, low fruit and vegetable intake, and/or lack of physical activity. Further, cancer-causing infections, such as human papillomavirus (HPV) and hepatitis, are responsible for approximately 30% of cancer cases in low- and lower-middle-income countries.

[0005] The treatments for cancers vary with the type of cancer and how advanced it is. Conventional treatments for cancers include surgery, radiation therapy, and chemotherapy. However, these treatments usually cause a variety of complications and side effects, such as infection, blood clots, bleeding, nausea and vomiting, diarrhea, nerve or muscle damage, incontinence, and/or sex and fertility issues. Immunotherapy provides an alternative strategy for cancer treatment that aims at specifically stimulating the immune response of a subject against cancer cells via blocking immune checkpoints or enhancing the ability of immune cells (e.g., T cells or B cells) to target and destroy cancer cells. Unfortunately, serious adverse effects caused by immunotherapy-mediated overstimulation or non-specific toxicity have been reported in cancer patients, including neurotoxicity, cytokine release syndrome (CRS), allergy, organ inflammation, and autoimmune disorders.

[0006] In view of the foregoing, there is a continuing interest in developing a novel method and/or agent for treating cancers.

SUMMARY

[0007] The following presents a simplified summary of the disclosure in order to provide a basic understanding to the reader. This summary is not an extensive overview of the disclosure, and it does not identify key/critical elements of the present invention or delineate the scope of the present invention. Its sole purpose is to present some concepts disclosed herein in a simplified form as a prelude to the more detailed description that is presented later.

[0008] As embodied and broadly described herein, the first aspect of the disclosure is directed to a recombinant antibody or a fragment thereof (*e.g.*, single-chain variable fragment, scFv). The recombinant antibody or antibody fragment comprises in its structure, a heavy chain variable (VH) domain and a light chain variable (VL) domain, in which the VH domain comprises a first heavy chain complementarity determining region (CDR-H1), a second heavy chain CDR (CDR-H2) and a third heavy chain CDR (CDR-H3); and the VL domain comprises a first light chain CDR (CDR-L1), a second light chain CDR (CDR-L2) and a third light chain CDR (CDR-L3).

[0009] According to some embodiments of the present disclosure, the CDR-H1, CDR-H2 and CDR-H3 respectively comprise the amino acid sequences of "GYXFTDY" (SEQ ID NO: 1), "DTSDSY" (SEQ ID NO: 2), and "GDY", in which the "X" residue at position 3 of SEQ ID NO: 1 is isoleucine (I) or threonine (T). In the embodiments, the CDR-L1, CDR-L2 and CDR-L3 respectively comprise the amino acid sequences of "QSLLESDGKTY" (SEQ ID NO: 3), "LVS", and "CQGTHFPWT" (SEQ ID NO: 4).

[0010] According to certain embodiments of the present disclosure, the recombinant antibody is designated as 16G2, in which the CDR-H1, CDR-H2 and CDR-H3 respectively comprise the amino acid sequences of "GYIFTDY" (SEQ ID NO: 5), "DTSDSY" (SEQ ID NO: 2), and "GDY"; and the CDR-L1, CDR-L2 and CDR-L3 respectively comprise the amino acid sequences of "QSLLESDGKTY" (SEQ ID NO: 3), "LVS", and "CQGTHFPWT" (SEQ ID NO: 4).

[0011] Depending on desired purpose, the antibody 16G2 may be produced in the form of a murine antibody (*i.e.*, both variable and constant domains of the antibody are derived from mouse), a chimeric antibody (*i.e.*, variable and constant domains of the antibody are respectively derived from mouse and human) or a humanized antibody (*i.e.*, the framework sequences of variable domains of the antibody are modified in accordance with antibody variants produced naturally in human).

[0012] According to one embodiment, the antibody 16G2 is produced in the form of a murine antibody, in which the VH and VL domains respectively comprise the amino acid sequences at least 85% identical to SEQ ID NOs: 7 and 8. In one exemplary embodiment, the VH and VL

domains of the murine antibody 16G2 respectively comprise the amino acid sequences 100% identical to SEQ ID NOs: 7 and 8, *i.e.*, the VH and VL domains of the murine antibody 16G2 respectively comprise the amino acid sequences of SEQ ID NOs: 7 and 8.

[0013] According to another embodiment, the antibody 16G2 is produced in the form of a chimeric antibody, in which the VH and VL domains respectively comprise the amino acid sequences at least 85% identical to SEQ ID NOs: 7 and ; preferably, the VH and VL domains of the chimeric antibody 16G2 respectively comprise the amino acid sequences of SEQ ID NOs: 7 and 8. In this embodiment, the chimeric antibody 16G2 further comprises a heavy chain constant (CH) and a light chain constant (CL) domains derived from human, in which the CH and CL domains respectively comprise the amino acid sequences at least 85% identical to SEQ ID NOs: 18 and 19; preferably, the CH and CL domains of the chimeric antibody 16G2 respectively comprise the amino acid sequences of SEQ ID NOs: 18 and 19.

[0014] According to still another embodiment, the antibody 16G2 is produced in the form of a humanized antibody, in which the VH domain comprises the amino acid sequence at least 85% identical to SEQ ID NO: 9, 10, 11 or 12; and the VL domain comprises the amino acid sequence at least 85% identical to SEQ ID NO: 13, 14 or 15. In one exemplary embodiment, the VH domain of the humanized antibody 16G2 comprises the amino acid sequence of SEQ ID NO: 9, 10, 11 or 12; and the VL domain of the humanized antibody 16G2 comprises the amino acid sequence of SEQ ID NO: 13, 14 or 15. In one specific embodiment, the VH domain of the humanize antibody 16G2 comprises the amino acid sequence of SEQ ID NO: 11; and the VL domain of the humanize antibody 16G2 comprises the amino acid sequence of SEQ ID NO: 13.

[0015] According to some embodiments of the present disclosure, the recombinant antibody is designated as 5B3, in which the CDR-H1, CDR-H2 and CDR-H3 respectively comprise the amino acid sequences of “GYTFTDY” (SEQ ID NO: 6), “DTSDSY” (SEQ ID NO: 2), and “GDY”; and the CDR-L1, CDR-L2 and CDR-L3 respectively comprise the amino acid sequences of “QSLLESDGKTY” (SEQ ID NO: 3), “LVS”, and “CQGTHFPWT” (SEQ ID NO: 4). As could be appreciated, the antibody 5B3 may be produced in the form of a murine antibody, a chimeric antibody or a humanized antibody in accordance with desired purpose.

[0016] According to one embodiment, the antibody 5B3 is produced in the form of a murine antibody, in which the VH and VL domains respectively comprise the amino acid sequences at least 85% identical to SEQ ID NOs: 16 and 17. In one exemplary embodiment, the VH and VL domains of the murine antibody 5B3 respectively comprise the amino acid sequences of SEQ ID NOs: 16 and 17.

[0017] According to another embodiment, the antibody 5B3 is produced in the form of a

chimeric antibody, in which the VH and VL domains respectively comprise the amino acid sequences at least 85% identical to SEQ ID NOs: 16 and 17; preferably, the VH and VL domains of the chimeric antibody 5B3 respectively comprise the amino acid sequences of SEQ ID NOs: 16 and 17. In this embodiment, the chimeric antibody 5B3 further comprises a CH and a CL domains derived from human, wherein the CH and CL domains respectively comprise the amino acid sequences at least 85% identical to SEQ ID NOs: 18 and 19; preferably, the CH and CL domains of the chimeric antibody 5B3 respectively comprise the amino acid sequences of SEQ ID NOs: 18 and 19.

[0018] Also disclosed therein are the uses of the antibody of the present disclosure in the preparation of a medicament or a pharmaceutical composition for treating cancers in a subject. The medicament or pharmaceutical composition comprises the antibody or antibody fragment in accordance with any embodiment or example of the present disclosure; and, optionally, a pharmaceutically acceptable carrier.

[0019] Another aspect of the present disclosure pertains to a method of treating a cancer in a subject. The method comprises administering to the subject an effective amount of the antibody, antibody fragment, medicament, or pharmaceutical composition of the present disclosure.

[0020] According to some embodiments, the cancer is a parathyroid hormone-related protein (PTHrP)-positive cancer (*i.e.*, the cancer expressing or secreting PTHrP); for example, gastric cancer, lung cancer, bladder cancer, breast cancer, pancreatic cancer, renal cancer, colorectal cancer, cervical cancer, ovarian cancer, brain tumor, prostate cancer, hepatocellular carcinoma, melanoma, esophageal carcinoma, multiple myeloma, or head and neck squamous cell carcinoma.

[0021] The subject treatable with the present recombinant antibody, medicament, pharmaceutical composition and/or method is a mammal; preferably, a human.

[0022] Many of the attendant features and advantages of the present disclosure will become better understood with reference to the following detailed description considered in connection with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] The present description will be better understood from the following detailed description read in light of the accompanying drawings briefly discussed below.

[0024] Fig. 1 depicts the effect of BGM-2121 on inhibiting the growth of BxPC-3 cancer cell according to Example 1.3 of the present disclosure.

[0025] Fig. 2 depicts the effect of BGM-2121 on inhibiting the migration of HARA-B cancer cell according to Example 1.3 of the present disclosure. IgG: an isotype antibody serving as a negative control in the study. * $p < 0.05$; ** $p < 0.01$.

[0026] Fig. 3 depicts the therapeutic effect of murine antibody 16G2 on non-small cell lung cancer (NSCLC) according to Example 2.1 of the present disclosure; Panel (A): Tumor volume of mice administered with specified treatment; Panel (B): Serum calcium level of mice administered with specified treatment. IgG: an isotype antibody serving as negative control in the study. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns (not significant): ≥ 0.05 .

[0027] Fig. 4 depicts the therapeutic effect of murine antibody 16G2 on pancreatic cancer according to Example 2.1 of the present disclosure; Panel (A): Tumor volume of mice administered with specified treatment; Panel (B): Serum calcium level of mice administered with specified treatment. IgG: an isotype antibody serving as a negative control in the study. * $p < 0.05$; ** $p < 0.01$.

[0028] Fig. 5 depicts the therapeutic effect of humanized antibody BGM-2121 on BxPC3 subcutaneous tumor model according to Example 2.2 of the present disclosure; Panel (A): Tumor volume of mice administered with hIgG or BGM-2121 twice per week, once per week, or once every two weeks; Panel (B): Tumor volume of mice administered with hIgG or BGM-2121 once every three weeks; Panel (C): Probability of survival of mice administered with hIgG or BGM-2121 once every three weeks. hIgG: human IgG isotype antibody serving as a negative control in the study. * $p < 0.05$; *** $p < 0.001$.

[0029] Fig. 6 depicts the therapeutic effect of humanized antibody BGM-2121 on BxPC3 orthotopic tumor model according to Example 2.2 of the present disclosure; Panel (A): Tumor weight of mice administered with specified treatment; Panel (B): Serum creatine phosphokinase (CPK) level of mice administered with specified treatment. hIgG: human IgG isotype antibody serving as a negative control in the study. * $p < 0.05$; ns: ≥ 0.05 .

[0030] Fig. 7 depicts the therapeutic effect of humanized antibody BGM-2121 on CFPAC-1 subcutaneous tumor model according to Example 2.2 of the present disclosure; Panel (A): Tumor volume of mice administered with specified treatment; Panel (B): Tumor weight of mice administered with specified treatment. Arrows indicated the day of antibody (Ab) injection. hIgG: human IgG isotype antibody serving as a negative control in the study. * $p < 0.05$.

DETAILED DESCRIPTION OF THE INVENTION

[0031] The detailed description provided below in connection with the appended drawings is

intended as a description of the present examples and is not intended to represent the only forms in which the present example may be constructed or utilized. The description sets forth the functions of the example and the sequence of steps for constructing and operating the example. However, the same or equivalent functions and sequences may be accomplished by different examples.

[0032] **I. DEFINITION**

[0033] For convenience, certain terms employed in the specification, examples and appended claims are collected here. Unless otherwise defined herein, scientific and technical terminologies employed in the present disclosure shall have the meanings that are commonly understood and used by one of ordinary skill in the art. Also, unless otherwise required by context, it will be understood that singular terms shall include plural forms of the same and plural terms shall include the singular. Specifically, as used herein and in the claims, the singular forms “a” and “an” include the plural reference unless the context clearly indicates otherwise. Also, as used herein and in the claims, the terms “at least one” and “one or more” have the same meaning and include one, two, three, or more.

[0034] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in the respective testing measurements. Also, as used herein, the term “about” generally means within 10%, 5%, 1%, or 0.5% of a given value or range. Alternatively, the term “about” means within an acceptable standard error of the mean when considered by one of ordinary skill in the art. Other than in the operating/working examples, or unless otherwise expressly specified, all of the numerical ranges, amounts, values and percentages such as those for quantities of materials, durations of times, temperatures, operating conditions, ratios of amounts, and the likes thereof disclosed herein should be understood as modified in all instances by the term “about”. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the present disclosure and attached claims are approximations that can vary as desired. At the very least, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

[0035] The term “antibody” (Ab) is used in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multi-specific or multivalent antibodies (*e.g.*, bi-specific antibodies), chimeric antibodies, humanized antibodies and antibody fragments so long as they exhibit the desired biological

activity. The term “antibody fragment” or “the fragment of an antibody” refers to a portion of a full-length antibody, generally the antigen binding or variable domain (*i.e.*, VH and VL domains) of a full-length antibody. Examples of the antibody fragment include fragment antigen-binding (Fab), Fab', F(ab')₂, single-chain variable fragment (scFv), diabody, linear antibody, single-chain antibody molecule, and multi-specific antibody formed from antibody fragments.

[0036] The term “complementarity determining region” (CDR) used herein refers to the hypervariable region of an antibody molecule that forms a surface complementary to the three-dimensional surface of a bound antigen. Proceeding from N-terminus to C-terminus, each of the antibody heavy and light chains comprises three CDRs (CDR-1, CDR-2 and CDR-3). An antigen combining site, therefore, includes a total of six CDRs that comprise three CDRs in the variable domain of a heavy chain (*i.e.*, CDR-H1, CDR-H2 and CDR-H3), and three CDRs in the variable domain of a light chain (*i.e.*, CDR-L1, CDR-L2 and CDR-L3).

[0037] The “variable domain” of an antibody refers to the amino-terminal domains of heavy or light chain of the antibody. These domains are generally the most variable parts of an antibody and contain the antigen-binding sites. The term “variable” refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called complementarity-determining regions (CDRs) or hypervariable regions both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., Sequences of Proteins of Immunological Interest, Fifth Edition, National Institute of Health, Bethesda, Md. (1991)).

[0038] As discussed herein, minor variations in the amino acid sequences of antibodies are contemplated as being encompassed by the presently disclosed and claimed inventive concept(s), providing that the variations in the amino acid sequence maintain at least 85% sequence identity, such as at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% sequence identity. The antibody of the present disclosure may be modified specifically to alter a feature of the antibody unrelated to its physiological activity. For example, certain amino acid residues in the framework (FR) region of the antibody can be

changed and/or deleted without affecting the physiological activity of the antibody in this study (*i.e.*, its ability to treat cancers). In particular, conservative amino acid replacements are contemplated. Conservative replacements are those that take place within a family of amino acid residues that are related in their side chains. Genetically encoded amino acid residues are generally divided into families: (1) acidic = aspartate, glutamate; (2) basic = lysine, arginine, histidine; (3) nonpolar = alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar = glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. More preferred families are: serine and threonine are aliphatic-hydroxy family; asparagine and glutamine are an amide-containing family; alanine, valine, leucine and isoleucine are an aliphatic family; and phenylalanine, tryptophan, and tyrosine are an aromatic family. For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the binding or properties of the resulting molecule, especially if the replacement does not involve an amino acid residue within the antigen-binding sites, *i.e.*, CDRs. Whether an amino acid change results in a functional peptide can readily be determined by assaying the specific activity of the peptide derivative. Fragments or analogs of proteins/peptides can be readily prepared by those of ordinary skill in the art. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains.

[0039] “Percentage (%) sequence identity” is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percentage sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, sequence comparison between two amino acid sequences was carried out by computer program Blastp (protein-protein BLAST) provided online by Nation Center for Biotechnology Information (NCBI). The percentage amino acid sequence identity of a given amino acid sequence A to a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has a certain % amino acid sequence identity to a given amino acid sequence B) is calculated by the formula as follows:

$$\frac{X}{Y} \times 100$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program BLAST in that program's alignment of A and B, and where Y is the total number of amino acid residues in A or B, whichever is shorter.

[0040] As used herein, the term “treat,” “treating” and “treatment” are interchangeable, and encompasses partially or completely preventing, ameliorating, mitigating and/or managing a symptom, a secondary disorder or a condition associated with cancers. The term “treating” as used herein refers to application or administration of the antibody of the present disclosure to a subject, who has a symptom, a secondary disorder or a condition associated with cancers, with the purpose to partially or completely alleviate, ameliorate, relieve, delay onset of, inhibit progression of, reduce severity of, and/or reduce incidence of one or more symptoms, secondary disorders or features associated with cancers. Symptoms, secondary disorders, and/or conditions associated with cancers include, but are not limited to, hypercalcemia (the calcium level in the blood is above normal), nausea, vomiting, loss of appetite, constipation, fatigue, muscle weakness, increased thirst, bone pain or broken bones, swelling or lump, bleeding, cough, fever, night sweats, coma and pain. Treatment may be administered to a subject who exhibits only early signs of such symptoms, disorder, and/or condition for the purpose of decreasing the risk of developing the symptoms, secondary disorders, and/or conditions associated with cancers. Treatment is generally “effective” if one or more symptoms or clinical markers are reduced as that term is defined herein. Alternatively, a treatment is “effective” if the progression of a symptom, disorder or condition is reduced or halted.

[0041] The term “effective amount” as referred to herein designate the quantity of a component which is sufficient to yield a desired response. For therapeutic purposes, the effective amount is also one in which any toxic or detrimental effects of the component are outweighed by the therapeutically beneficial effects. An effective amount of an agent is not required to cure a disease or condition but will provide a treatment for a disease or condition such that the onset of the disease or condition is delayed, hindered or prevented, or the disease or condition symptoms are ameliorated. The effective amount may be divided into one, two, or more doses in a suitable form to be administered at one, two or more times throughout a designated time period. The specific effective or sufficient amount will vary with such factors as the particular condition being treated, the physical condition of the patient (*e.g.*, the patient's body mass, age, or gender), the type of mammal or animal being treated, the duration of the treatment, the nature of concurrent therapy (if any), and the specific formulations employed and

the structure of the compounds or its derivatives. Effective amount may be expressed, for example, in grams, milligrams or micrograms or as milligrams per kilogram of body weight (mg/Kg). Alternatively, the effective amount can be expressed in the concentration of the active component (*e.g.*, the antibody of the present disclosure), such as molar concentration, mass concentration, volume concentration, molality, mole fraction, mass fraction and mixing ratio. Persons having ordinary skills could calculate the human equivalent dose (HED) for the medicament (such as the present antibody) based on the doses determined from animal models. For example, one may follow the guidance for industry published by US Food and Drug Administration (FDA) entitled “Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers” in estimating a maximum safe dosage for use in human subjects.

[0042] The terms “subject” refers to an animal including the human species that is treatable by the antibody, medicament, pharmaceutical composition and/or method of the present invention. The term “subject” is intended to refer to both the male and female gender unless one gender is specifically indicated.

[0043] **II. DESCRIPTION OF THE INVENTION**

[0044] *(i) Antibodies-murine mAb 16G2 and murine mAb 5B3*

[0045] The subject invention aims at treating diseases, especially PTHrP-related diseases (*e.g.*, cancers), in a subject, as well as providing pharmaceutical preparations for such purpose.

[0046] Accordingly, the first aspect of present disclosure is directed to two monoclonal antibodies (mAbs) respectively designated as “16G2” and “5B3”. According to embodiments of the present disclosure, each of the present mAbs exhibits a binding affinity to and a neutralizing effect on parathyroid hormone-related protein (PTHrP), a hormone acting as an endocrine, autocrine, paracrine and intracrine regulator to mediate cancer growth, progression and metastasis; and the administration of the present mAb is capable of treating cancers and PTHrP-induced hypercalcemia.

[0047] According to some embodiments of the present disclosure, the present mAb is produced by immunization method (*i.e.*, immunizing animals with specific peptides).

[0048] In general, the polypeptide (*i.e.*, PTHrP polypeptide) can be synthesized by commonly used methods such as t-BOC or FMOC protection of alpha-amino groups. Both methods involve stepwise syntheses whereby a single amino acid is added at each step starting from the C terminus of the peptide. Polypeptides of the invention can also be synthesized by the well-known solid phase peptide synthesis methods.

[0049] Then, the antibody can be produced by immunizing a host animal, such as a mouse, a rat, or a rabbit, with the synthetic polypeptide. The immunization may be performed in accordance with commonly adopted procedures. The immunization interval is not particularly limited. Immunization may be carried out at intervals of several days to several weeks, preferably one week, for 2-10 times, until a desired antibody titer is reached. For example, the host animals may be vaccinated by subcutaneously injecting with the synthetic polypeptide on weekly basis for 8 consecutive weeks.

[0050] After the final immunization, splenic cells and regional lymph nodes are removed. Blood samples are taken regularly after immunization and subject to centrifugation to separate sera. The resultant sera are then subject to measurement of antibody titers by any suitable method, which includes, but is not limited to, enzyme linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), or radio immunoassay (RIA). In one preferred example, antibody titers are measured by ELISA. Then, final immunization is given to those animals showing high antibody titers to the synthetic polypeptide. Antibody-producing cells are prepared from splenic cells and regional lymph nodes or the like of the immunized animals. In the preparation of antibody-producing cells, it is preferably to remove tissue debris and erythrocytes as much as possible. Commercial erythrocyte remover may be used to this purpose. Alternatively, a buffer ammonium chloride and Tris may be prepared and used. The thus prepared antibody-producing cells should be immediately fused with immortal cells such as myeloma cells to produce hybridoma cells, which semi-eternally continue to proliferate while producing antibodies. Commonly available cell strain derived from an animal such as mouse may be used. A preferable cell strain to be used in this invention should not survive in HAT selection medium, which contains hypoxanthine, thymidine and aminopterin; and should survive there only when fused with antibody-producing cells. Examples of myeloma cells include, but are not limited to, mouse myeloma cell line (such as myeloma FO cells) and human myeloma cell line (such as Karpas 707H). Cell fusion is usually carried out by mixing splenic cells or lymph node cells with a commercial available myeloma cells in the presence of a cell-fusion promoter, such as polyethylene glycol (PEG) having an average molecular weight from about 200 to 20,000 daltons or the like. Alternatively, cell fusion may be carried out in a commercial cell fusion device utilizing electric stimulation such as electroporation. After the fusion, the resultant cells are then diluted and cultured in HAT medium.

[0051] Hybridomas of interest are then selected from the fused cells. The fused cells surviving cultured in HAT medium would form colonies. The supernatant of each culture well is then collected and examine for the presence or absence of antibody titers to the polypeptide.

As a method of confirmation, ELISA, EIA or RIA may be used. Once antibody-positive wells are identified, cells are then cultured in a HT medium, which merely contains does not contain hypoxanthine and thymidine, without aminopterin. After culturing for a while, antibody titers in the culture supernatant are confirmed again. Cells that are finally selected are then subject to cloning to obtain single cells. Clones that exhibit high specificity to the polypeptide are selected, and are proliferated to some extent to establish hybridomas.

[0052] The mAbs produced by the hybridomas may be isolated or prepared by any known method. For example, antibodies may be prepared from cultured supernatant obtained by culturing hybridomas in a medium with low serum concentration. Alternatively, hybridomas may be injected into abdominal cavities of animals and the resultant abdominal dropsies are collected to prepare antibodies. Antibodies may be purified or isolated by methods that employ affinity column, gel filtration chromatography, ion exchange chromatography or the like. Any of these known methods may be appropriately selected or used in combination.

[0053] In structure, each of the thus-produced mAbs 16G2 and 5B3 comprises three CDRs in the VH domain thereof (*i.e.*, CDR-H1, CDR-H2, and CDR-H3), and three CDRs in the VL domain thereof (*i.e.*, CDR-L1, CDR-L2, and CDR-L3).

[0054] According to some embodiments of the present disclosure, the CDR-H1, CDR-H2, and CDR-H3 of mAb 16G2 respectively comprise the amino acid sequences of “GYIFTDY” (SEQ ID NO: 5), “DTSDSY” (SEQ ID NO: 2), and “GDY”; and the CDR-L1, CDR-L2, and CDR-L3 of mAb 16G2 respectively comprise the amino acid sequences of “QSLLESDGKTY” (SEQ ID NO: 3), “LVS”, and “CQGTHFPWT” (SEQ ID NO: 4).

[0055] As an example, the amino acid sequences of the VH and VL domains of mAb 16G2 are respectively provided as SEQ ID NOs: 7 and 8, described below, in which the CDRs (*i.e.*, the CDR-H1, CDR-H2 and CDR-H3 of VH domain, and the CDR-L1, CDR-L2 and CDR-L3 of VL domain) are marked in bold, in sequence.

[0056] SEQ ID NO: 7 (VH domain of mAb 16G2)

QVQLQQPGAELGMPGASVKMSCKAS**GYIFTDY**WMHWVKQRPGQGLEWIGAI**DTSDS**
YSSYNQKFQGKATLTVDSSSTAYMQLSSLTSEDSAVYYCTL**GDY**WGQGTTTLTVSS

[0057] SEQ ID NO: 8 (VL domain of mAb 16G2)

DVVMVTQTPLTSLVTIGQPASMSCKSS**QSLLESDGKTY**LNWLLQRPGQSPKRLIY**LVSKL**
DSGVPDRFTGSGSGTDFTLKISRVEAEDLGVIYCC**CQGTHFPWT**FGGGTKLEIK

[0058] Since the binding affinity and specificity of an antibody are mainly determined by the CDR sequences thereof, as could be appreciated, the framework (FR) sequences of the VH and

VL domains may vary (*e.g.*, being substituted by conserved or non-conserved amino acid residues) without affecting the binding affinity and/or specificity of the present antibody. Preferably, the FR sequence is conservatively substituted by one or more suitable amino acid(s) with similar properties; for example, the substitution of leucine (an nonpolar amino acid residue) by isoleucine, alanine, valine, proline, phenylalanine, or tryptophan (another nonpolar amino acid residue); the substitution of aspartate (an acidic amino acid residue) by glutamate (another acidic amino acid residue); or the substitution of lysine (an basic amino acid residue) by arginine or histidine (another basic amino acid residue).

[0059] Based on the conservative substitution, a skilled artisan may substitute the amino acid residue(s) of the FR sequences of the VH and VL domains of mAb 16G2 without affecting the activity and/or effect of mAb 16G2 (*i.e.*, neutralizing PTHrP and treating cancers). Accordingly, the antibody comprising substituted amino acid(s) in its FR sequences of VH and VL domains are intended to be included within the scope of the present disclosure. According to certain embodiments, the VH domain of mAb 16G2 comprises the amino acid sequence at least 85% (*i.e.*, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) identical to SEQ ID NO: 7, and the VL domain of mAb 16G2 comprises the amino acid sequence at least 85% (*i.e.*, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) identical to SEQ ID NO: 8. According to some preferred embodiments, the VH and VL domains of mAb 16G2 respectively comprise the amino acid sequences at least 90% identical to SEQ ID NOs: 7 and 8. More preferably, the VH and VL domains of mAb 16G2 respectively comprise the amino acid sequences at least 95% identical to SEQ ID NOs: 7 and 8.

[0060] According to some embodiments of the present disclosure, the CDR-H1, CDR-H2, and CDR-H3 of mAb 5B3 respectively comprise the amino acid sequences of “GYTFTDY” (SEQ ID NO: 6), “DTSDSY” (SEQ ID NO: 2), and “GDY”; and the CDR-L1, CDR-L2, and CDR-L3 of mAb 5B3 respectively comprise the amino acid sequences of “QSLLESDGKTY” (SEQ ID NO: 3), “LVS”, and “CQGTHFPWT” (SEQ ID NO: 4).

[0061] As an example, the amino acid sequences of the VH and VL domains of mAb 5B3 are respectively provided as SEQ ID NOs: 16 and 17, described below, in which the CDRs (*i.e.*, the CDR-H1, CDR-H2 and CDR-H3 of VH domain, and the CDR-L1, CDR-L2 and CDR-L3 of VL domain) are marked in bold, in sequence.

[0062] SEQ ID NO: 16 (VH domain of mAb 5B3)

QVQLQQPGAELVMPGASVKMSCKAS**GYTFTDY**WMHWVKQRPGQGLEWIGAL**DTSDS**
YASYNQRFK GKATLTVDASSSTAYMQLSSLTSEDSAVYYCTL**GDY**WGQGTTTLTVSS

[0063] SEQ ID NO: 17 (VL domain of mAb 5B3)

DVVMQTQTPLTSLVTFGQPASISCKSSQSLLES~~SDG~~KTYLNWLLQRPQGSPKRLIYLVSKL
DSGVPDRFTGSGSGTDFTLKISRVEAEDLGVYYCC~~QGTH~~FPWTFGGGTKLEIK

[0064] As described above, the FR sequences of the VH and VL domains may vary (*e.g.*, being substituted by conserved or non-conserved amino acid residues) without affecting the binding affinity and/or specificity of the present antibody, and a skilled artisan may substitute the amino acid residue(s) of the FR sequences of the VH and VL domains of mAb 5B3 without affecting the activity and/or effect of mAb 5B3 (*i.e.*, neutralizing PTHrP and treating cancers). Accordingly, the antibody comprising substituted amino acid(s) in its FR sequences of VH and VL domains are intended to be included within the scope of the present disclosure. According to certain embodiments, the VH domain of mAb 5B3 comprises the amino acid sequence at least 85% (*i.e.*, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) identical to SEQ ID NO: 16, and the VL domain of mAb 5B3 comprises the amino acid sequence at least 85% (*i.e.*, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) identical to SEQ ID NO: 17. According to some preferred embodiments, the VH and VL domains of mAb 5B3 respectively comprise the amino acid sequences at least 90% identical to SEQ ID NOs: 16 and 17. More preferably, the VH and VL domains of mAb 5B3 respectively comprise the amino acid sequences at least 95% identical to SEQ ID NOs: 16 and 17.

[0065] As could be appreciated, the present mAb (*i.e.*, mAb 16G2 or 5B3) may alternatively be produced by DNA cloning based the amino acid sequences disclosed above. Specifically, a skilled artisan may produce a DNA construct that comprises the nucleotide sequences encoding the VH and VL sequences of the present mAb, followed by transfecting the DNA construct into suitable host cells, such as *E. Coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells or myeloma cells that do not produce immunoglobulin proteins, to synthesize the desired mAb in the recombinant host cells.

[0066] Depending on intended purpose, the present mAb may be produced in the form of an immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM), immunoglobulin D (IgD) or immunoglobulin E (IgE).

[0067] **(ii) Antibodies-chimeric mAb 16G2 and chimeric mAb 5B3**

[0068] Each of the mAbs (including mAbs 16G2 and 5B3) described in *Section (i)* of the present disclosure is useful in producing a chimeric antibody, *i.e.*, the antibody having variable domains from its original species (*e.g.*, a mouse) and constant domains from another species (*e.g.*,

a human), so as to reduce the immunogenicity of the antibody in a subject.

[0069] Accordingly, the present disclosure also provides two chimeric antibodies respectively derived from murine mAbs 16G2 and 5B3, in which the constant domains of the murine mAbs are replaced with those of a human antibody.

[0070] According to certain embodiments, the VH domain of chimeric mAb 16G2 comprises the amino acid sequence of SEQ ID NO: 7 as described in *Section (i)* of the present disclosure, and the VL domain of chimeric mAb 16G2 comprises the amino acid sequence of SEQ ID NO: 8.

[0071] According to some embodiments, the VH domain of chimeric mAb 5B3 comprises the amino acid sequence of SEQ ID NO: 16 as described in *Section (i)* of the present disclosure, and the VL domain of chimeric mAb 5B3 comprises the amino acid sequence of SEQ ID NO: 17.

[0072] In these embodiments, each of the chimeric mAbs 16G2 and 5B3 comprises a CH domain of SEQ ID NO: 18 linked to its VH domain, and a CL domain of SEQ ID NO: 19 linked to its VL domain. The amino acid sequences of the CH and CL are provided below.

[0073] SEQ ID NO: 18 (CH domain of chimeric mAbs)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSGSSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[0074] SEQ ID NO: 19 (CL domain of chimeric mAbs)

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0075] As described, the constant domains and the FR sequences of the VH and VL domains may vary (*e.g.*, being substituted by conserved or non-conserved amino acid residues) without affecting the binding affinity and/or specificity of the present antibody, and a skilled artisan may substitute the amino acid residue(s) of the constant domains and/or the FR sequences of the VH and VL domains of present chimeric mAb without affecting its activity and/or effect (*i.e.*, neutralizing PTHrP and treating cancers). Accordingly, the antibody comprising substituted amino acid(s) in its constant domains and/or FR sequences of VH and VL domains are intended to be included within the scope of the present disclosure.

[0076] Depending on intended purpose, the chimeric mAb may be produced in the form of an

IgG, IgA, IgM, IgD or IgE.

[0077] *(iii) Antibodies-humanized mAb 16G2*

[0078] Alternatively, each of the mAbs (including mAbs 16G2 and 5B3) described in *Section (i)* of the present disclosure is useful in producing a humanized antibody, *i.e.*, the amino acid sequences of a non-human antibody (*e.g.*, a murine antibody) are modified to increase its similarity to the antibody variant produced naturally in humans, so as to minimize the immunogenicity of the antibody in a human subject.

[0079] Accordingly, the present disclosure further provides different humanized VH and VL sequences, including humanized VH1 (SEQ ID NO: 9), humanized VH2 (SEQ ID NO: 10), humanized VH3 (SEQ ID NO: 11), humanized VH4 (SEQ ID NO: 12), humanized VL1 (SEQ ID NO: 13), humanized VL2 (SEQ ID NO: 14), and humanized VL3 (SEQ ID NO: 15). The amino acid sequences of the humanized VH and VL domains are provided below, in which the CDRs (*i.e.*, the CDR-H1, CDR-H2 and CDR-H3 of VH domain, and the CDR-L1, CDR-L2 and CDR-L3 of VL domain) are marked in bold, in sequence.

[0080] SEQ ID NO: 9 (humanized VH1 domain of mAb 16G2)

QVQLVQSGAEVKKPGASVKVSCKASGYIF**TDYWMHWVRQAPGQGLEWMGAIDTSDS**
YSSYNQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCT**LG**DYWGQGTTVTVSS

[0081] SEQ ID NO: 10 (humanized VH2 domain of mAb 16G2)

QVQLVQSGAEVKKPGASVKVSCKASGYIF**TDYWMHWVRQAPGQGLEWIGAI**DTSDSY
SSYNQKFQGRATMTVDTSTSTVYMELSSLRSEDTAVYYCT**LG**DYWGQGTTVTVSS

[0082] SEQ ID NO: 11 (humanized VH3 domain of mAb 16G2)

QVQLVQSGAEVKKPGASVKVSCKASGYIF**TDYWMHWVKQAPGQGLEWIGAI**DTSDSY
SSYNQKFQGRATMTVDTSTSTVYMELSSLRSEDTAVYYCT**LG**DYWGQGTTVTVSS

[0083] SEQ ID NO: 12 (humanized VH4 domain of mAb 16G2)

QVQLVQSGAEVKKPGASVKVSCKASGYIF**TDYWMHWVKQAPGQGLEWIGAI**DTSDSY
SSYNQKFQGRATMTVDESTSTVYMELSSLRSEDTAVYYCT**LG**DYWGQGTTVTVSS

[0084] SEQ ID NO: 13 (humanized VL1 domain of mAb 16G2)

DVVMQTQSPLSLPVT**LGQPASISCKSSQSLLES**DGKTYLNWFQQRPGQSPRRLIYLVSKLD
SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**CQGTHFPWTF**GGG**TKLEIK**

[0085] SEQ ID NO: 14 (humanized VL2 domain of mAb 16G2)

DVVMQTQSPLSLPVT**LGQPASISCKSSQSLLES**DGKTYLNWLLQRPQSPRRLIYLVSKLD
SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**CQGTHFPWTF**GGG**TKLEIK**

[0086] SEQ ID NO: 15 (humanized VL3 domain of mAb 16G2)

DVVMTQSPLSLPVTLGQPASMSCKSSQSLLES~~SDGKTYLNWLLQ~~RPGQSPKRLIYLVSKL
DSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCC~~QGTHFPWTF~~GGGKLEIK

[0087] As described, the FR sequences of the VH and VL domains may vary (e.g., being substituted by conserved or non-conserved amino acid residues) without affecting the binding affinity and/or specificity of the present antibody, and a skilled artisan may substitute the amino acid residue(s) of the FR sequences of the VH and VL domains of present humanized mAb without affecting its activity and/or effect (*i.e.*, neutralizing PTHrP and treating cancers). Accordingly, the antibody comprising substituted amino acid(s) in the FR sequences of the humanized VH and VL domains are intended to be included within the scope of the present disclosure.

[0088] According to one embodiment, the humanized mAb designated as “humanized mAb 16G2-1” comprises the VH1 domain (SEQ ID NO: 9) and VL1 domain (SEQ ID NO: 13). According to another embodiment, the humanized mAb designated as “humanized mAb 16G2-2” comprises the VH1 domain (SEQ ID NO: 9) and VL2 domain (SEQ ID NO: 14). According to another embodiment, the humanized mAb designated as “humanized mAb 16G2-3” comprises the VH1 domain (SEQ ID NO: 9) and VL3 domain (SEQ ID NO: 15).

[0089] According to one embodiment, the humanized mAb designated as “humanized mAb 16G2-4” comprises the VH2 domain (SEQ ID NO: 10) and VL1 domain (SEQ ID NO: 13). According to another embodiment, the humanized mAb designated as “humanized mAb 16G2-5” comprises the VH2 domain (SEQ ID NO: 10) and VL2 domain (SEQ ID NO: 14). According to another embodiment, the humanized mAb designated as “humanized mAb 16G2-6” comprises the VH2 domain (SEQ ID NO: 10) and VL3 domain (SEQ ID NO: 15).

[0090] According to one embodiment, the humanized mAb designated as “humanized mAb 16G2-7” comprises the VH3 domain (SEQ ID NO: 11) and VL1 domain (SEQ ID NO: 13). According to another embodiment, the humanized mAb designated as “humanized mAb 16G2-8” comprises the VH3 domain (SEQ ID NO: 11) and VL2 domain (SEQ ID NO: 14). According to another embodiment, the humanized mAb designated as “humanized mAb 16G2-9” comprises the VH3 domain (SEQ ID NO: 11) and VL3 domain (SEQ ID NO: 15).

[0091] According to one embodiment, the humanized mAb designated as “humanized mAb 16G2-10” comprises the VH4 domain (SEQ ID NO: 12) and VL1 domain (SEQ ID NO: 13). According to another embodiment, the humanized mAb designated as “humanized mAb 16G2-11” comprises the VH4 domain (SEQ ID NO: 12) and VL2 domain (SEQ ID NO: 14).

According to another embodiment, the humanized mAb designated as “humanized mAb 16G2-12” comprises the VH4 domain (SEQ ID NO: 12) and VL3 domain (SEQ ID NO: 15).

[0092] Depending on intended purpose, the humanized mAb may be produced in the form of an IgG, IgA, IgM, IgD or IgE.

[0093] *(iv) Medicament or pharmaceutical composition comprising the present mAb*

[0094] According to some embodiments of the present disclosure, the present mAb (including the murine mAb, chimeric mAb and humanized mAb respectively described in *Sections (i)-(iii)* of the present disclosure) exhibits a binding affinity and neutralizing activity toward PTHrP. Accordingly, another aspect of the present disclosure pertains to a medicament or a pharmaceutical composition for the treatment of a cancer, especially the cancer caused by and/or associated with PTHrP. The medicament or pharmaceutical composition comprises the mAb of the present disclosure, or its fragment (*e.g.*, scFv); and optionally, a pharmaceutically acceptable carrier.

[0095] Generally, the mAb or antibody fragment of this invention is present at a level of about 0.1% to 99% by weight, based on the total weight of the medicament or a pharmaceutical composition. In some embodiments, the mAb or antibody fragment of this invention is present at a level of at least 1% by weight, based on the total weight of the medicament or a pharmaceutical composition. In certain embodiments, the mAb or antibody fragment is present at a level of at least 5% by weight, based on the total weight of the medicament or a pharmaceutical composition. In still other embodiments, the mAb or antibody fragment is present at a level of at least 10% by weight, based on the total weight of the medicament or a pharmaceutical composition. In still yet other embodiments, the mAb or antibody fragment is present at a level of at least 25% by weight, based on the total weight of the medicament or a pharmaceutical composition.

[0096] The pharmaceutically acceptable carrier may be any pharmaceutically acceptable material or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, that is useful in carrying or transporting the active agents (*e.g.*, the present mAb or antibody fragment) from one organ, or portion of the body, to another organ, or portion of the body. The carrier must be “acceptable” in the sense of being compatible with the other ingredients of the formulation, and is selected to minimize any degradation of the active agent and to minimize any adverse side effects in the subject. Depending on desired purposes, the medicament or a pharmaceutical composition of the present disclosure may further comprise one or more pharmaceutically-acceptable additives, including binder, flavoring, buffering agent, thickening agent, coloring agent, anti-oxidant, diluent, stabilizer, buffer, emulsifier, dispersing

agent, suspending agent, antiseptic and the like.

[0097] The choice of a pharmaceutically acceptable carrier to be used in conjunction with the present mAb/antibody fragment is basically determined by the way the medicament or a pharmaceutical composition being administered. The medicament or a pharmaceutical composition of the present invention may be administered to a subject via subcutaneous, intravenous, or intramuscular injection.

[0098] The medicament or a pharmaceutical composition for administration by injection may be prepared in a sterile aqueous or non-aqueous solution, suspension, and emulsion. Examples of the non-aqueous solution include, but are not limited to, propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Illustrative examples of the aqueous solution include water, alcoholic solution, emulsion, or suspension, such as saline and buffered media. Common parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose, sodium chloride, lactated Ringer's, or fixed oils; whereas intravenous vehicles often include fluid and nutrient replenishes, electrolyte replenishes (such as those based on Ringer's dextrose), and the like.

[0099] *(v) Uses of the present antibody, pharmaceutical composition, and medicament*

[0100] Another aspect of the present disclosure pertains to a method of treating a cancer in a subject. The method comprises administering to the subject an effective amount of the mAb, antibody fragment, pharmaceutical composition, or medicament in accordance with any aspect, embodiment or example of the present disclosure.

[0101] According to some embodiments of the present disclosure, the cancer is a PTHrP-positive cancer, *i.e.*, the cancer expressing or secreting PTHrP; examples of cancers treatable with the present mAb, antibody fragment, pharmaceutical composition, medicament and/or method include, but are not limited to, gastric cancer, lung cancer, bladder cancer, breast cancer, pancreatic cancer, renal cancer, colorectal cancer, cervical cancer, ovarian cancer, brain tumor, prostate cancer, hepatocellular carcinoma, melanoma, esophageal carcinoma, multiple myeloma, or head and neck squamous cell carcinoma. In one exemplary embodiment, the cancer is the lung cancer. In another exemplary embodiment, the cancer is the pancreatic cancer.

[0102] According to some embodiments, the subject is a mouse, in which 0.1 to 100 mg/Kg of the present mAb or antibody fragment is administered to the subject, such as 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49,

50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 mg/kg. Preferably, about 1 mg/kg to 50 mg/kg of the present mAb or antibody fragment is administered to the subject. More preferably, about 5 mg/kg to 20 mg/kg of the present mAb or antibody fragment is administered to the subject. According to one working example, about 10 mg/kg of the present mAb or antibody fragment is sufficient to elicit a therapeutic effect in the subject (*i.e.*, inhibiting tumor growth, and alleviating or ameliorating the symptoms caused by cancer, such as hypercalcemia).

[0103] A skilled artisan may readily determine the human equivalent dose (HED) of the present mAb or antibody fragment, based on the doses determined from animal studies provided in working examples of this application. Accordingly, the effective amount of the present mAb/antibody fragment suitable for use in a human subject may be in the range of 10 µg/kg to 10 mg/kg body weight for human; such as 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990 µg/kg, or 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 mg/kg. Preferably, the HED is about 100 µg/kg to 5 mg/kg body weight. The dose can be administered in a single aliquot, or alternatively in more than one aliquot. The skilled artisan or clinical practitioner may adjust the dosage or regime in accordance with the physical condition of the patient or the severity of the diseases.

[0104] Depending on desired purpose, the present mAb, antibody fragment, pharmaceutical composition, and/or medicament may be administered to the subject 1 or 2 times every week, once every 2 weeks, once every 3 weeks, once every 4 weeks, once every 5 weeks, once every 6 weeks, once every 7 weeks, once every 8 weeks, or longer. The progress of this therapy is easily monitored by conventional techniques and assays. As could be appreciated, the dosing regimen can vary over time. According to one exemplary embodiment, the present mAb is administered to the subject twice every week. According to another exemplary embodiment, the present mAb is administered to the subject once every week. According to still another exemplary embodiment, the present mAb is administered to the subject once every 2 weeks. According to an further exemplary embodiment, the present mAb is administered to the subject once every 3 weeks.

[0105] The present mAb, antibody fragment, pharmaceutical composition, and/or medicament

may be administered to the subject by a route selected from the group consisting of nasal, topical, transmucosal, and parenteral administration, in which the parenteral administration is any of subcutaneous, intramuscular, intravenous, or intraperitoneal injection.

[0106] As would be appreciated, the present method can be applied to the subject, alone or in combination with additional therapies that have some beneficial effects on the prevention or treatment of cancers. Depending on the intended/therapeutic purpose, the present method can be applied to the subject before, during, or after the administration of the additional therapies.

[0107] According to certain embodiments of the present disclosure, the administration of the present mAb, antibody fragment, pharmaceutical composition, or medicament inhibits tumor growth. According to some embodiments of the present disclosure, the administration of the present mAb, antibody fragment, pharmaceutical composition, or medicament reduces the calcium level in the blood (*e.g.*, serum) of the subject.

[0108] Basically, the subject treatable by the present method is a mammal, for example, human, mouse, rat, guinea pig, hamster, monkey, swine, dog, cat, horse, sheep, goat, cow, and rabbit. Preferably, the subject is a human.

[0109] The following Examples are provided to elucidate certain aspects of the present invention and to aid those of skilled in the art in practicing this invention. These Examples are in no way to be considered to limit the scope of the invention in any manner. Without further elaboration, it is believed that one skilled in the art can, based on the description herein, utilize the present invention to its fullest extent. All publications cited herein are hereby incorporated by reference in their entirety.

EXAMPLE

[0110] **Materials and Methods**

[0111] *Cell culture*

[0112] Rat osteosarcoma cell line UMR-106 was cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Human lung cancer cell line HARA-B and human pancreatic cell line BxPC-3 were cultured in RPMI medium supplemented with 10% FBS. Human pancreatic cell line CFPAC-1 were cultured in Iscove's Modified Dulbecco's Medium (IMDM) medium supplemented with 10% FBS.

[0113] *Preparation of murine mAbs 16G2 and 5B3*

[0114] The murine monoclonal antibodies were produced via mice immunization. Specifically, the BALB/c mice were immunized with an *Escherichia coli* (*E. coli*)-expressed

fusion protein comprising a PTHrP peptide and a polyhistidine-tag (His-tag) fused to the C-terminus of the PTHrP peptide. Post-immunization, B cells were extracted from the spleens of the immunized mice, followed by fusing with myeloma cells. The titers of the antibodies secreted by the thus-produced hybridomas were determined by ELISA so as to identify the hybridoma clones (clones 16G2 and 5B3) that expressed the antibodies exhibiting binding affinity and specificity towards PTHrP. The selected clones 16G2 and 5B3 were then cultured to facilitate antibody production. The antibodies isolated from the supernatant of the culture medium of clones 16G2 and 5B3 were purified, and then subjected to sequence analysis and activity assays.

[0115] The thus-produced antibodies were respectively designated as “murine mAb 16G2” and “murine mAb5B3”, in which the VH and VL sequences of the murine antibodies were summarized in Table 1.

[0116] Table 1 VH and VL sequences of murine mAbs 16G2 and 5B3

Name	Amino acid sequence	SEQ ID NO
mAb 16G2 VH	QVQLQQPGAELGMPGASVKMSCKASGYIFTDYWMHWVKQRPG QGLEWIGAI DTSDSYSSYNQKFQ GKATLTVDSSSTAYMQLSSLTS EDSAVYYCTLG DYWGQG TTLTVSS	7
mAb 16G2 VL	DVVMQTPLTSLVITGQPASMSCKSS QSLLES DGKTYLNWLLQRP GQSPKRLIYLVSKLDSGVPDRFTGSGSGTDFTLKISRVEAEDLGVY YCC QGT HFPTFGGGTKLEIK	8
mAb 5B3 VH	QVQLQQPGAELVMPGASVKMSCKASGYTFTDYWMHWVKQRPG QGLEWIGAL DTSDSYASYNQRFK GKATLTVDASSSTAYMQLSSLT SEDSAVYYCTLG DYWGQG TTLTVSS	16
mAb 5B3 VL	DVVMQTPLTSLVITFGQPASISCKSS QSLLES DGKTYLNWLLQRP GQSPKRLIYLVSKLDSGVPDRFTGSGSGTDFTLKISRVEAEDLGVY YCC QGT HFPTFGGGTKLEIK	17

* The CDR sequences (including CDR-H1, CDR-H2 and CDR-H3 of VH domain, and CDR-L1, CDR-L2 and CDR-L3 of VL domain) were marked in bold, in sequence.

[0117] *Preparation of chimeric mAbs 16G2 and 5B3*

[0118] The nucleotide sequence encoding the VH and VL domains of murine mAbs 16G2 and 5B3 were respectively cloned into the expression vector pcDNA3.4, which contained a constant domain of human IgG. The vector containing both non-human variable and human constant domains were transfected into host cells EXPICHO-STM for expression. The chimeric monoclonal antibodies were isolated from the supernatant of the culture medium, followed by purification and sequence analysis.

[0119] The thus-produced chimeric mAb comprised the VH and VL domains of murine mAb (*i.e.*, the VH and VL domains of murine mAb 16G2 or murine mAb 5B3), and the constant

domains of a human antibody, in which the CL domain comprises the amino acid sequence of SEQ ID NO: 18, and the CH domain comprises the amino acid sequence of SEQ ID NO: 19.

[0120] *Preparation of humanized mAb 16G2*

[0121] The mouse-derived framework regions within the VH and VL domains of the chimeric mAb were first identified, and replaced by human framework regions while preserving critical binding areas. Structural and functional assessments were employed to ensure the successful humanization of these regions. Subsequently, the modified variable domains were expressed in host cells EXPICHO-STM and optimized for maximum production of the humanized mAb. After purification, the humanized mAbs were subjected to sequence analysis and activity assays.

[0122] Four humanized VH domains (including VH1, VH2, VH3 and VH4 domains) and three humanized VL domains (including VL1, VL2 and VL3 domains) were produced in this study. The amino acid sequences of the humanized VH and VL domains were summarized in Table 2.

[0123] Table 2 Amino acid sequences of humanized VH and VL domains

Name	Amino acid sequence	SEQ ID NO
VH1	QVQLVQSGAEVKKPGASVKVSCKASGYIFTDYWMHWVRQAPGQ GLEWMGAIDTSDSYSSYNQKFQGRVTMTTRDTSTSTVYMELSSLRS EDTAVYYCTLGDYWGQGTTVTVSS	9
VH2	QVQLVQSGAEVKKPGASVKVSCKASGYIFTDYWMHWVRQAPGQ GLEWIGAIDTSDSYSSYNQKFQGRATMTVDTSTSTVYMELSSLRS EDTAVYYCTLGDYWGQGTTVTVSS	10
VH3	QVQLVQSGAEVKKPGASVKVSCKASGYIFTDYWMHWVKQAPG QGLEWIGAIDTSDSYSSYNQKFQGRATMTVDTSTSTVYMELSSLR SEDTAVYYCTLGDYWGQGTTVTVSS	11
VH4	QVQLVQSGAEVKKPGASVKVSCKASGYIFTDYWMHWVKQAPG QGLEWIGAIDTSDSYSSYNQKFQGRATMTVDESTSTVYMELSSLR SEDTAVYYCTLGDYWGQGTTVTVSS	12
VL1	DVVMTQSPSLSPVTILGQPASISCKSSQSLLES DGKTYLNWFQQR GQSPRRLIYLVSKLDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVY YCCQGT HFPWTFGGGTKLEIK	13
VL2	DVVMTQSPSLSPVTILGQPASISCKSSQSLLES DGKTYLNWLLQR GQSPRRLIYLVSKLDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVY YCCQGT HFPWTFGGGTKLEIK	14
VL3	DVVMTQSPSLSPVTILGQPASMSCKSSQSLLES DGKTYLNWLLQR PGQSPKRLIYLVSKLDSGVPDRFSGSGSGTDFTLKISRVEAEDVGV YCCQGT HFPWTFGGGTKLEIK	15

* The CDR sequences (including CDR-H1, CDR-H2 and CDR-H3 of VH domain, and CDR-L1, CDR-L2 and CDR-L3 of VL domain) were marked in bold, in sequence.

[0124] The humanized mAbs containing the humanized VH and VL domains were respectively designated as “humanized mAb 16G2-1” (the mAb comprising humanized VH1 and

VL1 sequences), “humanized mAb 16G2-2” (the mAb comprising humanized VH1 and VL2 sequences), “humanized mAb 16G2-3” (the mAb comprising humanized VH1 and VL3 sequences), “humanized mAb 16G2-4” (the mAb comprising humanized VH2 and VL1 sequences), “humanized mAb 16G2-5” (the mAb comprising humanized VH2 and VL2 sequences), “humanized mAb 16G2-6” (the mAb comprising humanized VH2 and VL3 sequences), “humanized mAb 16G2-7” (the mAb comprising humanized VH3 and VL1 sequences; also named as “BGM-2121” in the study), “humanized mAb 16G2-8” (the mAb comprising humanized VH3 and VL2 sequences), “humanized mAb 16G2-9” (the mAb comprising humanized VH3 and VL3 sequences), “humanized mAb 16G2-10” (the mAb comprising humanized VH4 and VL1 sequences), “humanized mAb 16G2-11” (the mAb comprising humanized VH4 and VL2 sequences), and “humanized mAb 16G2-12” (the mAb comprising humanized VH4 and VL3 sequences).

[0125] *Surface plasmon resonance (SPR)*

[0126] The affinity of antigen to antibodies were measured using BIACORE™ SPR Systems. In brief, antibodies were injected on the sensor chip and immobilized with mouse antibody capture kit as capture. Antigen was diluted into multiple concentrations (50, 25, 12.5, 6.25, 3.125, 1.5625 nM) and injected over the surface of flow cell 1 and 2 as association phase, followed by injecting HBS-EP+ running buffer (HEPES 10 mM, NaCl 150 mM, EDTA 3 mM, 0.005% TWEEN® 20) as dissociation phase. All the data were recorded at 25°C and processed using software.

[0127] *Enzyme linked immunosorbent assay (ELISA)*

[0128] The biotinylated PTHrP fragment containing the 34 N-terminal residues of human PTHrP (hereinafter as “PTHrP (1-34) peptide”) and biotinylated parathyroid hormone (PTH) fragment containing the 34 N-terminal residues of human PTH (hereinafter as “PTH (1-34) peptide”) were respectively diluted to a final concentration of 0.2 µg/mL, and then added to a 96-well plate. The plate was incubated at room temperature for 2 hours. After the washing and blocking steps, the diluted antibody was added to the wells, followed by incubating at room temperature for 2 hours. Next, a secondary antibody was added to the wells. After further washes, TMB (3,3',5,5'-Tetramethylbenzidine) substrate was added to induce color development. The reaction was halted using HCL, and the absorbance was measured at 450 nm with a reference at 570 nm. The resulting absorbance data was analyzed by software via nonlinear regression for determining the half maximal effective concentration (EC50).

[0129] *Cell based-neutralizing assay*

[0130] For cell-base cAMP neutralization assay, UMR-106 cells were seeded into a 96-well plate at a density of 1.5×10^4 cells per well and incubated at 37°C for 3 days. Then, the medium was removed and washed by serum-free medium and then cells were incubated with experimental medium (DMEM containing 10 mM HEPES and 0.5 mM isobutylmethylxanthine (IBMX)) at 37°C for 30 minutes. The experimental medium was removed, and recombinant PTHrP (1-34) peptide (20 ng/mL) with or without anti-PTHrP antibody (4x series dilution: from 2.5 µg/mL to 0.04 µg/mL) were added and then incubated at 37°C for 30 minutes. After incubation, the medium was aspirated and treated with 250 µl 0.1 M HCL at room temperature for 20 minutes. Cells were harvested and transferred to a V-bottom 96-well dish followed by centrifuged at 1,000 xg for 10 minutes at 4°C. The supernatant was transferred into a clean test tube. The absorbance was measured at a wavelength between 405 and 420 nm using an ELISA reader.

[0131] *In vitro neutralizing assay (competition ELISA)*

[0132] PTH1R protein (1 µg/mL) in coating buffer was added into ELISA plate and incubated overnight at 4°C, followed by washing with PBS-T washing buffer (1X PBS containing 0.05% TWEEN® 20). The protein coating was blocked by incubation with blocking buffer (washing buffer containing 2% (w/v) bovine serum albumin (BSA)) at 37°C for 1.5 hours. Biotinylated PTHrP was diluted to 0.5 µg/mL with dilution buffer (washing buffer containing 0.5% (w/v) BSA) and anti-PTHrP antibody was diluted to 40, 10, 2.5 and 0.625 µg/mL with dilution buffer. The mixture of biotinylated PTHrP and anti-PTHrP antibodies were added into the plate and incubated at 37°C for 1 hour. Next, 100 µL of streptavidin-HRP (0.1 µg/mL) working solution were added into the plate and incubated at 37°C for 1 hour, and then added 100 µL TMB substrate working solution to each well. After 10 minutes, 100 µL of 1N HCl were added to stop the reaction. The absorbance was measured at a wavelength 450 nm using an ELISA reader.

[0133] *Cell viability*

[0134] The number of viable cells treated with specified treatment (*i.e.*, 0, 25, 50, or 100 µg/mL of anti-PTHrP antibody) was determined by luminescent cell viability assay, a quantitative method based on the amount of adenosine triphosphate (ATP) present in the cells. In brief, 5×10^3 BxPC-3 cells were seeded in a 96-well plate, followed by incubating at 37°C for 24 hours. After removing the medium, 50 µl/well of RPMI supplemented with 5% FBS was added to the cells. The cells were incubated at 37°C for 2 hours (starvation period). The anti-PTHrP antibody or gemcitabine (serving as a positive control) was administered into the wells, and the cells were incubated at 37°C with 5% CO₂ for 72, 96 or 108 hours. Pre-warmed

luminescent reagent was added to the wells, and the luminescence was measured after incubation at room temperature for 10 minutes.

[0135] *Wound healing assay*

[0136] Human lung cancer cell line HARA-B cells (1×10^5 cells) were seeded into cell culture insert system, forming a confluent layer, and then incubated at 37°C with 5% CO₂ for 24 hours. After rinsing twice with 1X PBS, the cells were incubated in 70 µl of 2% FBS medium for 24 hours (starvation period). The PTHrP (1-34) peptide (10 µg/mL) and antibody (anti-PTHrP antibody or human-IgG, 100 µg/mL) were pre-complexed for 1 hour at room temperature. The cell culture insert system was removed, and the cells were washed twice with 1X PBS. The mixture of the PTHrP peptide and antibody was added to each dish. The cell images were captured using a microscope at 0, 12, 24, 32 and 48 hours.

[0137] *Animal models*

[0138] *(i) Treatment of non-small cell lung cancer by using murine mAb 16G2*

[0139] Four to six-week-old nude mice were used for xenograft tumor growth studies and the animals were acclimatized and housed under pathogen-free conditions. For the tumor growth assay, 5×10^6 HARA-B cells were suspended in 200 µL PBS and subcutaneously injected into the dorsal flanks of mice. Tumor volume was determined by measuring tumor length (L) and width (W) using calipers and calculated by the formula (length $\times$ width $\times$ width/2). After inoculation, mice were randomly divided into two groups to receive anti-PTHrP antibody (10 mg/kg) or control IgG by intraperitoneal (i.p.) three times per week. The serum was collected for calcium measurements.

[0140] *(ii) Treatment of pancreatic cancer by using murine mAb 16G2*

[0141] Four to six-week-old nude mice were used for xenograft tumor growth studies and the animals were acclimatized and housed under pathogen-free conditions. For the xenograft tumor growth assay, 5×10^6 BxPC-3 cells were suspended in 200 µL PBS and subcutaneously injected into the dorsal flanks of mice. Tumor volume was determined by measuring tumor length (L) and width (W) using calipers and calculated by the formula (length $\times$ width $\times$ width/2). After inoculation, mice were randomly divided into two groups to receive anti-PTHrP antibody (10 mg/kg) or control IgG by intraperitoneal (i.p.) injection three times per week.

[0142] *(iii) Treatment of pancreatic cancer by using humanized mAb 16G2-7 (BGM-2121)*

[0143] Four to six-week-old nude mice were used for xenograft tumor growth studies and the animals were acclimatized and housed under pathogen-free conditions. For the xenograft tumor growth assay, 5×10^6 BxPC-3 or CFPAC-1 cells were suspended in 200 µL PBS and

subcutaneously injected into the dorsal flanks of mice. Tumor volume was determined by measuring tumor length (L) and width (W) using calipers and calculated by the formula (length $\times$ width $\times$ width/2). After inoculation, mice were randomly divided into two groups to receive anti-PTHrP antibody (1, 3 or 10 mg/kg) or control hIgG by intravenous (i.v.) injection at specified time points. The serum was collected for calcium measurements. For intrasplenic tumor injection, an incision on the left ventral side was made under anesthesia. The spleen was gently pulled out and 2×10^6 BxPC-3 cells were slowly injected into the spleen. After inoculation, mice were randomly divided into two groups to receive anti-PTHrP antibody (10 mg/kg) or control hIgG by intravenous injection (i.v.) at specified time points. The serum was collected for calcium measurements.

[0144] *Statistical analysis*

[0145] All experiments repeated at least 3 times. The results were evaluated by Student's *t*-test. P value less than 0.05 was regarded as significant. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns (not significant): ≥ 0.05 .

[0146] **Example 1 Characterization of the present mAb**

[0147] **1.1 Binding affinity**

[0148] The binding activities of the present mAb to PTHrP were evaluated in this example. As described in "Materials and Methods" of the present disclosure, the present mAb (*i.e.*, the murine mAb, chimeric mAb or humanized mAb) and PTHrP antigen were respectively added to sensor chips, and the binding activity therebetween was determined by SPR. The results were summarized in Tables 3 and 4.

[0149] The data of Table 3 indicated that all tested mAb (including murine mAb 16G2, murine mAb 5B3, chimeric mAb 16G2 and chimeric mAb 5B3) recognized and bound to PTHrP, in which compared to the murine mAbs and reference Ab (*i.e.*, Chugai antibody, serving as a positive control), the chimeric antibodies exhibited higher binding affinity to PTHrP (the KD values of chimeric 16G2 mAb and chimeric 5B3 mAb were 1.15×10^{-10} and 2.08×10^{-10} , respectively).

[0150] Table 3 Antigen-binding affinity of specified mAbs

	Ligand	Analyte	k_a (1/Ms)	k_d (1/s)	KD (M)	Rmax (RU)	Chi ² (RU ²)
Mouse	16G2	peptide	2.14E+06	1.97E-03	9.17E-10	19.43	0.00866
	5B3	peptide	6.61E+05	4.38E-04	6.63E-10	13.04	0.00692
	Positive control (reference Ab)	peptide	1.14E+06	6.05E-04	5.29E-10	10.84	0.00975

Chimeric	16G2	peptide	2.64E+06	3.04E-04	1.15E-10	32.73	0.0155
	5B3	peptide	1.48E+06	3.07E-04	2.08E-10	38.36	0.0159
	Positive control (reference Ab)	peptide	2.88E+06	1.80E-03	6.25E-10	54.56	0.0481

Ka: The association rate constant.

Kd: The dissociation rate constant.

KD: The equilibrium dissociation constant; KD = kd/ka.

[0151] The data of Table 4 demonstrated the binding affinity of humanized mAbs (including humanized mAb 16G2-1 to humanized mAb 16G2-12) to PTHrP.

[0152] Table 4 Antigen-binding affinity of specified humanized mAbs

Ligand	Analyte	Chi ² (RU ²)	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)
Reference Ab	naïve peptide	1.70E-02	1.97E+06	6.03E-03	3.06E-09	46
Murine 16G2	naïve peptide	3.63E-02	2.99E+06	2.87E-04	9.60E-11	31.2
mAb 16G2-1	naïve peptide	2.80E-02	8.61E+05	1.27E-04	1.48E-10	42.2
mAb 16G2-2	naïve peptide	1.79E-02	1.28E+06	5.27E-04	4.13E-10	41
mAb 16G2-3	naïve peptide	4.07E-01	1.58E+06	1.72E-03	1.09E-09	42.1
mAb 16G2-4	naïve peptide	4.35E-02	1.08E+06	1.51E-04	1.39E-10	37.2
mAb 16G2-5	naïve peptide	1.04E-02	1.19E+06	6.04E-04	5.09E-10	44.7
mAb 16G2-6	naïve peptide	1.57E-02	1.55E+06	6.27E-04	4.04E-10	38
mAb 16G2-7 (BGM-2121)	naïve peptide	1.47E-02	1.18E+06	1.90E-04	1.60E-10	36
mAb 16G2-8	naïve peptide	1.97E-02	1.46E+06	2.50E-04	1.72E-10	41.5
mAb 16G2-9	naïve peptide	4.51E-02	2.37E+06	4.35E-04	1.84E-10	34.5
mAb 16G2-10	naïve peptide	1.10E-02	1.16E+06	1.02E-04	8.83E-11	49.5
mAb 16G2-11	naïve peptide	1.36E-02	1.42E+06	2.94E-04	2.08E-10	60.4
mAb 16G2-12	naïve peptide	1.36E-02	1.84E+06	1.68E-04	9.11E-11	38.1

Ka: The association rate constant. Kd: The dissociation rate constant. KD: The equilibrium dissociation constant; KD = kd/ka.

Reference Ab: Chugai antibody; serving as a positive control.

Murine 16G2: Murine mAb 16G2 comprising the VH domain of SEQ ID NO: 7 and the VL domain of SEQ ID NO: 8.

[0153] The binding affinities of the chimeric mAb 16G2 and mAb 16G2-7 (BGM-2121) toward PTHrP (1-34) peptide or PTH (1-34) peptide were further confirmed by ELISA. According to the result of Table 5, the chimeric mAb 16G2 and BGM-2121 recognized and bound to the PTHrP (1-34) peptide with equilibrium dissociation constant (KD) of 2.44 nM and 1.79 nM, respectively. It was noted that the chimeric mAb 16G2 as well as BGM-2121 exhibited no off-target binding to the PTH (1-34) fragment, the primary regulator of calcium and phosphate metabolism in bone and kidney.

[0154] Table 5 Binding affinity (EC50) of specified antibodies towards PTHrP (1-34) or PTH (1-34) peptide

Peptide	Chimeric mAb 16G2	BGM-2121	anti-hPTH**
PTH (1-34)	> 1 mM	> 1 mM	3.06 nM
PTHrP (1-34)	2.44 nM	1.79 nM	> 1 mM

** Anti-hPTH: a commercial antibody developed by Phoenix Pharmaceuticals that exhibited a high binding affinity toward human PTH (1-34) peptide; serving as a control group in the study.

[0155] 1.2 *In vitro* activity

[0156] In this example, the bio-activity of the present mAb was analyzed by neutralization assay. The results were summarized in Tables 6 and 7.

[0157] Cell-based neutralization assay was performed using the rat osteosarcoma cell line UMR-106, which expresses endogenous PTH1 receptor. All tested mAb (including murine mAb 16G2, murine mAb 5B3, chimeric mAb 16G2 and chimeric mAb 5B3) inhibited the PTHrP-induced cAMP production in UMR-106 cells (data not shown). According to the results of Table 6, both chimeric mAb 16G2 (with an EC₅₀ of 0.81 nM) and BGM-2121 (with an EC₅₀ of 0.89 nM) exhibited high effectiveness in selectively inhibiting PTHrP (1-34) peptide-induced cAMP production. Importantly, both chimeric mAb 16G2 and BGM-2121 exhibited no neutralizing activity against PTH (1-34) peptide-induced cAMP production (with an EC₅₀ > 40 nM).

[0158] Table 6 Neutralizing activity (EC50) of specified antibodies by cell-based neutralization assay

Peptide	Chimeric mAb 16G2	BGM-2121
hPTH (1-34)	> 40 nM	> 40 nM
PTHrP (1-34)	0.81 nM	0.89 nM

[0159] The data of Table 7 indicated that each of the present humanized mAbs 16G2-1, 16G2-4, 16G2-7 (BGM-2121) and 16G2-10 was useful in neutralizing the activity of PTHrP antigen, in which the humanized 16G2-10 mAb exhibited the highest competition neutralizing activity ($IC_{50} = 1.36 \times 10^{-8}$; Table 7), and the humanized 16G2-7 mAb exhibited the highest cell-base neutralizing activity ($IC_{50} = 8.926 \times 10^{-10}$; Table 7).

[0160] Table 7 The neutralizing the activity of specified humanized mAbs

Item	Heavy chain	Light chain	Back mutation	KD (M)	Competition Neutralizing activity (M)	Cell-base Neutralizing activity (M)
Name	VH	VL	0	1.15E-10	2.25E-08	8.147e-010
mAb 16G2-1	VH1 (h-2 back)		2	1.29E-10	1.70E-08	1.206e-009
mAb 16G2-4	VH2 (h-5 back)	VL1 (h-0 back)	5	1.16E-10	1.95E-08	1.328e-009
mAb 16G2-7	VH3 (h-6 back)		6	8.79E-11	1.54E-08	8.926e-010
mAb 16G2-1	VH4 (h-7 back)		7	1.03E-10	1.36E-08	9.389e-010
R	Positive control (reference Ab)			3.06E-09	3.07E-08	7.852e-009

[0161] The data of Table 8 further confirmed that BGM-2121 was capable of binding to biotin-labeled PTHrP (1-34) peptide and competed with PTH1R binding. The result suggested that BGM-2121 exerted a neutralizing activity by interfering with the interaction between the biotin-labeled PTHrP (1-34) peptide and PTH1R receptor. According to the results of Table 8, both the chimeric mAb 16G2 antibody (with an EC_{50} of 22.5 nM) and BGM-2121 (with an EC_{50} of 15.4 nM) exhibited potent inhibition of human PTHrP (1-34) peptide.

[0162] Table 8 Neutralizing activity (EC_{50}) of specified antibodies by competition ELISA-based assay

Name	Chimeric mAb 16G2	BGM-2121
PTHrP (1-34)	22.5 nM	15.4 nM

[0163] These results demonstrated that each of the present mAb (including murine mAb, chimeric mAb and humanized mAb) exhibited PTHrP-binding affinity, and may serve as a neutralizing antibody to treat cancers (especially PTHrP-positive cancers) via inhibiting the activity of PTHrP.

[0164] **1.3 Inhibitory effect on cancer cells**

[0165] As described in Materials and Methods, BxPC-3 cells were treated with the present anti-PTHrP antibody (BGM-2121) or gemcitabine (serving as a positive control) for 72, 96 or 108 hours, and the cell viability was then determined by luminescent cell viability assay. The data of Fig. 1 demonstrated that BGM-2121 exhibited a dose-dependent effect on the viability of BxPC-3 cells.

[0166] The wound healing assay further demonstrated the inhibitory effect of BGM-2121 on cell migration, in which the treatment of the PTHrP (1-34) peptide significantly enhanced the migration ability of HARA-B cells, while the co-incubation of 100 µg/ml BGM-2121 inhibited the migration of the PTHrP (1-34) peptide-stimulated cells (24 hours post-treatment; Fig. 2), suggesting that BGM-2121 possessed the potential to inhibit the migration ability of HARA-B cells.

[0167] **Example 2 Anti-tumor effect of the present mAb**

[0168] **2.1 Murine mAb 16G2 treatment**

[0169] Two animal models were used in the example to evaluate the therapeutic effect of the murine mAb 16G2 on cancers. As described in “Materials and Methods” of the present disclosure, lung cancer cell line (*i.e.* HARA-B) and pancreatic cell line (*i.e.* BxPC-3) were respectively injected into the dorsal flanks of mice, followed by the treatment of the murine mAb 16G2 or control antibody IgG. The results were respectively depicted in Figs. 3 to 4.

[0170] Compared to the control antibody IgG, the administration of murine mAb 16G2 significantly inhibited the tumor growth in HARA-B model (NSCLC) (Panel (A) of Fig. 3). Further, the serum calcium levels were significantly reduced in HARA-B bearing mice treated with murine 16G2 antibody as compared to that in HARA-B bearing mice treated with control antibody IgG (Panel (B) of Fig. 3).

[0171] The data of Fig. 4 confirmed the anti-tumor effect of murine mAb 16G2, in which compared to the control antibody IgG, the administration of murine 16G2 significantly inhibited the tumor growth in BxPC-3 model (pancreatic cancer) (Panel (A) of Fig. 4) and reduced the serum calcium levels in BxPC-3 bearing mice (Panel (B) of Fig. 4). There was no significant difference in body weight between the mice administrated with control antibody IgG and murine mAb 16G2 (data not shown).

[0172] **2.2 BGM-2121 treatment**

[0173] The anti-tumor effect of humanized mAb BGM-2121 was examined in this example. In the subcutaneous tumor model, nude mice were subcutaneously injected in the flank with BxPC3 cells, and then intravenously administered with 10 mg/kg of BGM-2121 or isotype hIgG

antibody twice per week, once per week, once every 2 weeks, or once every 3 weeks. The data indicated that compared to the hIgG treatment, the treatment of BGM-2121 once per week or once every 2 weeks significantly inhibited tumor growth (Panel (A) of Fig. 5). According to the results, the administration of BGM-2121 (10 mg/kg) once per week resulted in a notable 30% reduction in tumor volume as compared to the mice treated with hIgG (data not shown). Further, the treatment of BGM-2121 (10 mg/kg) once every 3 weeks greatly inhibit the tumor volume (about 65% reduction) and significantly improved the overall survival of tumor-bearing mice (about 30% increase) as compared to the hIgG control group (Panels (B) and (C) of Fig. 5).

[0174] In the orthotopic tumor model, BxPC3 cells were injected into the spleen of mice to establish a xenograft model simulating pancreatic liver metastasis. Subsequently, these mice were intravenously administered with 10 mg/kg of BGM-2121 or isotype hIgG antibody on days 7, 14, 21, 28 and 35. The analysis of tumor weights revealed notable differences in situ, indicating significant growth suppression of the pancreas hyperplasia in the mice treated with BGM-2121 (about 58% inhibition) as compared to the hIgG Ab treatment group (Panel (A) of Fig. 6). Further, the serum CPK levels were reduced in BxPC-3 bearing mice treated with BGM-2121 as compared to the hIgG control group (Panel (B) of Fig. 6).

[0175] In addition to the BxPC3 tumor model, the anti-tumor effect of the present BGM-2121 was further confirmed by CFPAC-1 tumor model. CFPAC-1 cells was subcutaneously injected to the flank of nude mice, followed by intravenous administration of 10 mg/kg BGM-2121 or hIgG, once a week for a total of 4 weeks. The data of Fig. 7 indicated that the administration of the present BGM-2121 significantly inhibited tumor growth (about 40% inhibition; Panel (A) of Fig. 7) and tumor weight (Panel (B) of Fig. 7) as compared to the hIgG treatment.

[0176] In conclusion, the present disclosure provides two novel murine mAbs, 16G2 and 5B3, each of which is useful in producing a chimeric antibody or a humanized antibody. According to example of the present disclosure, the present mAbs exhibited binding affinity and neutralizing activity toward PTHrP, and may serve as a therapeutic agent for treating cancers, especially PTHrP-positive cancers.

[0177] It will be understood that the above description of embodiments is given by way of example only and that various modifications may be made by those with ordinary skill in the art. The above specification, examples, and data provide a complete description of the structure and use of exemplary embodiments of the invention. Although various embodiments of the invention have been described above with a certain degree of particularity, or with reference to one or more individual embodiments, those with ordinary skill in the art could make numerous

alterations to the disclosed embodiments without departing from the spirit or scope of this invention.

[0178] Insofar as the background, descriptions and examples above and the accompanying drawing disclose any additional subject matter that is not within the scope of what is claimed below, the inventions are not dedicated to the public and the right to file one or more applications to claim such additional inventions is reserved.

WHAT IS CLAIMED IS:

1. A recombinant antibody or a fragment thereof, comprising a heavy chain variable (VH) domain and a light chain variable (VL) domain, wherein the VH domain comprises a first heavy chain complementarity determining region (CDR-H1), a second heavy chain CDR (CDR-H2) and a third heavy chain CDR (CDR-H3), and the VL domain comprises a first light chain CDR (CDR-L1), a second light chain CDR (CDR-L2) and a third light chain CDR (CDR-L3), wherein
the CDR-H1, CDR-H2 and CDR-H3 respectively comprise the amino acid sequences of “GYXFTDY” (SEQ ID NO: 1), “DTSDSY” (SEQ ID NO: 2), and “GDY”; wherein X of SEQ ID NO: 1 is isoleucine (I) or threonine (T); and
the CDR-L1, CDR-L2 and CDR-L3 respectively comprise the amino acid sequences of “QSLLESDGKTY” (SEQ ID NO: 3), “LVS”, and “CQGTHFPWT” (SEQ ID NO: 4).
2. The recombinant antibody or the fragment thereof according to claim 1, wherein
the CDR-H1, CDR-H2 and CDR-H3 respectively comprise the amino acid sequences of “GYIFTDY” (SEQ ID NO: 5), “DTSDSY” (SEQ ID NO: 2), and “GDY”; and
the CDR-L1, CDR-L2 and CDR-L3 respectively comprise the amino acid sequences of “QSLLESDGKTY” (SEQ ID NO: 3), “LVS”, and “CQGTHFPWT” (SEQ ID NO: 4).
3. The recombinant antibody or the fragment thereof according to claim 2, wherein the VH and VL domains respectively comprise the amino acid sequences of SEQ ID NOs: 7 and 8.
4. The recombinant antibody or the fragment thereof according to claim 2, wherein
the VH domain comprises the amino acid sequence of SEQ ID NO: 9, 10, 11 or 12; and
the VL domain comprises the amino acid sequence of SEQ ID NO: 13, 14 or 15.
5. The recombinant antibody or the fragment thereof according to claim 4, wherein the VH and VL domains respectively comprise the amino acid sequences of SEQ ID NOs: 11 and 13.
6. The recombinant antibody or the fragment thereof according to claim 1, wherein
the CDR-H1, CDR-H2 and CDR-H3 respectively comprise the amino acid sequences of “GYTFTDY” (SEQ ID NO: 6), “DTSDSY” (SEQ ID NO: 2), and “GDY”; and
the CDR-L1, CDR-L2 and CDR-L3 respectively comprise the amino acid sequences of “QSLLESDGKTY” (SEQ ID NO: 3), “LVS”, and “CQGTHFPWT” (SEQ ID NO: 4).

7. The recombinant antibody or the fragment thereof according to claim 6, wherein the VH and VL domains respectively comprise the amino acid sequences of SEQ ID NOs: 16 and 17.
8. A pharmaceutical composition comprising the recombinant antibody of claim 1 or the fragment thereof, and a pharmaceutically acceptable excipient.
9. A method of treating a cancer in a subject, comprising administering to the subject an effective amount of the recombinant antibody of claim 1 or the fragment thereof, or the pharmaceutical composition of claim 8.
10. The method of claim 9, wherein the cancer is a parathyroid hormone-related protein (PTHrP)-expressing cancer.
11. The method of claim 10, wherein the cancer is gastric cancer, lung cancer, bladder cancer, breast cancer, pancreatic cancer, renal cancer, colorectal cancer, cervical cancer, ovarian cancer, brain tumor, prostate cancer, hepatocellular carcinoma, melanoma, esophageal carcinoma, multiple myeloma, or head and neck squamous cell carcinoma.
12. The method of claim 9, wherein the subject is a human.