POLYPEPTIDE AND USE THEREOF IN CONSTRUCTION OF BISPECIFIC ANTIBODY CONTAINING COMMON LIGHT CHAIN

Cross-Reference to Related Applications

This patent application claims the priority benefit of Chinese invention patent application No. CN202410331237.1, filed on March 22, 2024, the entire contents of which are incorporated herein for all purposes.

Technical Field

The present invention relates to the field of biomedicine, and in particular, relates to a peptide and use thereof as an antibody light chain, as well as antibodies against EGFR and/or c-Met comprising said peptide as a common light chain.

Background

Somatic mutations in the epidermal growth factor receptor (EGFR) are among the most common oncogenic drivers across non-small cell lung cancer (NSCLC), metastatic colorectal cancer, glioblastoma, head and neck cancer, pancreatic cancer, breast cancer or the like, and are particularly significant in non-small cell lung cancer. Although molecular targeted drugs against the EGFR signaling pathway have shown potent clinical efficacy, patients often develop drug resistance, and the resistance mechanisms are usually inevitable. For example, studies have found that non-small cell lung cancer cells with EGFR mutations acquire resistance to EGFR tyrosine kinase inhibitors (EGFR-TKIs) through multiple pathways, including activation of the c-Met receptor pathway.

Among these mechanisms, there is a close relationship between the c-Met receptor and EGFR resistance. c-Met is the receptor for hepatocyte growth factor (HGF) and possesses tyrosine kinase activity. The c-Met proto-oncogene bypasses the inhibited EGFR phosphorylation pathway through pathways such as ERBB3-PI3K-AKT and MAPK-ERK1/2, thereby producing an amplification effect. This amplified c-Met activates downstream signal transduction through a bypass route, avoiding the action of EGFR-TKIs, promoting continued cancer cell proliferation,

and ultimately resulting in resistance of patients to EGFR-TKIs. c-Met is normally expressed at low or undetectable levels in most normal tissues, but its high expression can be observed in tumor tissues such as lung cancer, liver cancer, pancreatic cancer, and thyroid cancer.

For dual-target therapy against EGFR and c-Met, the use of bispecific antibodies can effectively block signaling of both receptors simultaneously, inhibiting tumor growth and survival. At the same time, such antibodies can also enhance antibody-dependent cell-mediated cytotoxicity (ADCC), thereby increasing the potential to kill tumor cells. Studies have shown that such bispecific antibodies can simultaneously bind EGFR and c-Met and induce co-degradation of these two target receptors in various tumor cells, an effect that cannot be achieved by mAbs used alone or in combination. Because the mechanism of bispecific antibodies depends on co-expression of EGFR and c-Met, they have lower toxicity and greater therapeutic advantages compared to EGFR inhibitors alone.

Current conventional methods of producing bispecific antibodies usually need co-transfection of four plasmids to express two different heavy chains and two different light chains respectively. However, when these four polypeptide chains are assembled into antibodies, heavy-light chain mispairing may occur. Several strategies have been proposed in the art to address heavy-light chain mispairing in bispecific antibody assembly. Among these strategies, it has been proposed to use the same polypeptide as a common light chain to pair respectively with heavy chains of antibodies against two different target proteins (e.g., EGFR and c-Met), thereby constructing bispecific antibodies that have binding affinity and specificity for both target proteins. The resultant bispecific antibody is structurally similar to a conventional monoclonal antibody; furthermore, it follows that this polypeptide can also serve as a common light chain to pair with heavy chains from antibodies against more target proteins, thereby constructing antibodies with binding affinity and specificity for more different target proteins.

Summary of the Invention

The objective of the present invention is to provide a polypeptide that, as an antibody light chain variable region or light chain, can pair with a heavy chain variable region or heavy chain of an antibody with binding affinity and/or specificity for a certain target protein (antigen), or with two or even more heavy chain variable regions or heavy chains of antibodies that respectively have

binding affinity and/or specificity for different target proteins (antigens), to construct antibodies. Another objective of the present invention is to provide antibodies comprising said polypeptide as a light chain variable region or light chain, for example antibodies in the form of homodimers or heterodimers.

The technical solutions of the present invention are as follows.

In a first aspect, the present invention provides a polypeptide, the amino acid sequence of which comprises: the amino acid sequence set forth in SEQ ID NO: 40, the amino acid sequence set forth in SEQ ID NO: 41, and the amino acid sequence set forth in SEQ ID NO: 42.

SEQ ID NO: 40: SASSSVTFMH

SEQ ID NO: 41: DTSNLAS

SEQ ID NO: 42: QQWGDDPPYT

Further, the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 20.

SEQ ID NO: 20:

DIQLTQSPSTLSASVGDRVTITCSASSSVTFMHWYQQKPGTSPKRWIYDTSNLASGVPSRF
SGSGSGTEYTLTISSLQPDDFATYYCQQWGDDPPYTFGQGTRLEIK

Further, the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 29.

SEQ ID NO: 29:

DIQLTQSPSTLSASVGDRVTITCSASSSVTFMHWYQQKPGTSPKRWIYDTSNLASGVPSRF
SGSGSGTEYTLTISSLQPDDFATYYCQQWGDDPPYTFGQGTRLEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLS
KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

The polypeptides provided in the present invention, as antibody light chain variable regions or light chains, can respectively pair with heavy chain variable regions or heavy chains of antibodies with binding affinities and/or specificities for different target proteins (antigens), to construct antibodies, and said antibodies retain or have stronger binding affinities and/or specificities for the

target proteins (antigens) and corresponding biological activities, or the like.

The constructed antibodies may have any structural format, such as monoclonal antibodies or antibody formats such as scFv, dsFv, (dsFv)2, Fab, Fab', F(ab')2 or Fv. For example, said polypeptide, as an antibody light chain variable region or light chain, can pair with a heavy chain variable region or heavy chain of an antibody with binding affinity and/or specificity for a certain target protein (antigen), to construct an antibody that still has binding affinity and/or specificity for that target protein (antigen), that is, said antibody is a homodimeric antibody having two identical heavy chains and two identical light chains (a monospecific antibody). Alternatively, for example, said polypeptide, as an antibody light chain variable region or light chain, can pair with heavy chain variable regions or heavy chains of two antibodies that respectively have binding affinity and/or specificity for two target proteins (antigens), to construct an antibody that still has binding affinity and/or specificity for each of the two target proteins (antigens), that is, said antibody is a heterodimeric antibody having two different heavy chains and two identical light chains (a bispecific antibody). Even further, the polypeptides provided in the present invention can also be used to construct multispecific antibodies having binding affinities and/or specificities for more target proteins (antigens).

In a second aspect, the present invention provides a nucleic acid molecule comprising a nucleotide sequence encoding the polypeptide of the first aspect.

In a third aspect, the present invention provides use of the polypeptide of the first aspect or the nucleic acid molecule of the second aspect in the construction of an antibody.

Experiments have demonstrated that, as antibody light chains or light chain variable regions, the polypeptides provided in the first aspect of the present invention can pair with heavy chains or heavy chain variable regions of antibodies that have binding affinities and/or specificities for different target proteins (antigens), to construct new antibodies, and said antibodies retain (or even have stronger) binding affinities and/or specificities and biological activities for the target proteins (antigens), or the like.

According to embodiments of the present invention, the target protein may be epidermal growth factor receptor (EGFR) or hepatocyte growth factor receptor (c-Met or HGFR). In the context of the present invention, the epidermal growth factor receptor or hepatocyte growth factor receptor

may each be mammalian, primate or rodent, more preferably human EGFR or human c-Met (HGFR).

Correspondingly, as described above in the first aspect, the constructed antibodies may be homodimeric antibodies with two identical heavy chains and two identical light chains, or heterodimeric antibodies with two different heavy chains and two identical light chains. Said polypeptide may serve as a light chain or light chain variable region of an antibody.

In a fourth aspect, the present invention provides an antibody or antigen-binding fragment thereof, comprising the polypeptide provided in the first aspect of the present invention; wherein said polypeptide may serve as a light chain variable region or light chain of said antibody or antigen-binding fragment thereof.

In the context of the present invention, the antigen-binding fragment is any functional fragment of an antibody capable of specifically binding to a target protein; in the context of the present invention, the terms "a target protein" and "an antigen" can be used interchangeably.

The antibody or antigen-binding fragment thereof provided in the present invention may be an IgG-like antibody, or may be in an antibody format such as scFv, dsFv, (dsFv)2, Fab, Fab', F(ab')2 or Fv. In the present invention, the term "IgG-like antibody" means that the antibody or antigen-binding fragment thereof has two Fab arms and optional Fc regions, and is of a conventional monoclonal antibody (mAb) structure or similar structure. In the present invention, the term "Fab arm" refers to a Fab fragment (an antigen-binding fragment), consisting of a complete light chain (a variable region and a constant region) and a partial heavy chain structure (a variable region and a constant region fragment), with the light chain and heavy chain linked by a disulfide bond.

Accordingly, for example, the antibody or antigen-binding fragment thereof provided in the present invention comprises the amino acid sequences provided in the first aspect of the present invention in its light chain variable region as light chain CDRs; alternatively, the antibody or antigen-binding fragment thereof provided in the present invention comprises the amino acid sequence set forth in SEQ ID NO: 20 as the light chain variable region (VL). Alternatively, the antibody or antigen-binding fragment thereof provided in the present invention comprises the amino acid sequence set forth in SEQ ID NO: 29 as the light chain.

Further, the antibody or antigen-binding fragment thereof may further comprise a heavy chain variable region (VH), which forms a binding domain for a target protein together with the light chain variable region. According to embodiments of the present invention, the target protein may be epidermal growth factor receptor (EGFR) or hepatocyte growth factor receptor (c-Met or HGFR).

According to embodiments of the present invention, the antibody or antigen-binding fragment thereof comprises the following heavy chain CDRs (HCDR1, HCDR2 and HCDR3) and light chain CDRs (LCDR1, LCDR2 and LCDR3):

(1) HCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 36, HCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 38, and HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 39; and LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 40, LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 41, and LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 42;

(2) HCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 30, HCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 43, and HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 32; and LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 40, LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 41, and LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 42; or

(3) HCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 36, HCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 37, and HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 32; and LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 40, LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 41, and LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 42.

Further, the antibody or antigen-binding fragment thereof may comprise a heavy chain variable region and a light chain variable region respectively comprising:

(1) the amino acid sequence set forth in SEQ ID NO: 13, and the amino acid sequence set forth in SEQ ID NO: 14;

(2) the amino acid sequence set forth in SEQ ID NO: 19, and the amino acid sequence set forth in

SEQ ID NO: 20;

(3) the amino acid sequence set forth in SEQ ID NO: 15, and the amino acid sequence set forth in SEQ ID NO: 20; or

(4) the amino acid sequence set forth in SEQ ID NO: 17, and the amino acid sequence set forth in SEQ ID NO: 20.

The antibody or antigen-binding fragment thereof may be a murine antibody, a chimeric antibody or a humanized antibody; and may further comprise heavy chain constant regions and light chain constant regions. According to embodiments of the present invention, the heavy chain constant region may be a heavy chain constant region of an IgG1 or IgG4 antibody; the light chain constant region may be a light chain constant region of a kappa or lambda light chain. For example, the heavy chain constant region of the antibody comprises the amino acid sequence set forth in SEQ ID NO: 21 (hIgG1); and the light chain constant region of the antibody comprises the amino acid sequence set forth in SEQ ID NO: 22 (hkappa). Alternatively, the heavy chain constant region of the antibody comprises the amino acid sequence set forth in SEQ ID NO: 23 (hIgG1; Knob) or SEQ ID NO: 24 (hIgG1; Hole); and the light chain constant region of the antibody comprises the amino acid sequence set forth in SEQ ID NO: 22.

The antibody or antigen-binding fragment thereof provided in the present invention may have at least two combinations of VH + VL domains (specifically, two Fab arms), and said combinations of the domains may be identical to each other, thereby binding to the same target protein (antigen). In this case, the antibody or antigen-binding fragment thereof comprises, in its at least two combinations of VH + VL domains, identical amino acid sequence combinations selected from the group consisting of HCDR1 to HCDR3 and LCDR1 to LCDR3 provided in items (1) to (3) above, or comprises identical amino acid sequence combinations selected from the group consisting of the heavy and light chain variable regions provided in items (1) to (4) above. The antibody or antigen-binding fragment thereof further comprises heavy chain constant regions and light chain constant regions. For example, as described above, the heavy chain constant region comprises the amino acid sequence set forth in SEQ ID NO: 21; and the light chain constant region comprises the amino acid sequence set forth in SEQ ID NO: 22. Further, the antibody or antigen-binding fragment thereof has heavy chains and light chains, and is, for example, a monoclonal antibody

with two identical heavy chains and two identical light chains.

Alternatively, the at least two combinations of VH + VL domains (specifically, at least two Fab arms) of the antibody or antigen-binding fragment thereof provided in the present invention may be different, thereby binding to different target proteins (antigens). In this case, the antibody comprises, in its at least two combinations of VH + VL domains (specifically, at least two Fab arms), different amino acid sequence combinations selected from the group consisting of HCDR1 to HCDR3 and LCDR1 to LCDR3 provided in items (1) to (3) above, or comprises different amino acid sequence combinations selected from the group consisting of the heavy and light chain variable regions provided in items (1) to (4) above. In this case, it is preferred that the at least two combinations of VH + VL domains (specifically, at least two Fab arms) of the antibody or antigen-binding fragment thereof comprise identical light chain CDR sequence combinations, or comprise identical light chain VL sequences. For example, the at least two combinations of VH + VL domains (specifically, at least two Fab arms) each have: LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 40, LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 41, and LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 42. Further, for example, the two combinations of VH + VL domains (specifically, two Fab arms) each have a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 20.

According to embodiments of the present invention, the antibody or antigen-binding fragment thereof comprises:

(1) a first VH + VL domain combination (specifically, a Fab arm) binding to c-Met, said first VH + VL domain combination having: HCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 36, HCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 38, and HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 39; and LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 40, LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 41, and LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 42, and

a second VH + VL domain combination (specifically, a Fab arm) binding to EGFR, said second VH + VL domain combination having: HCDR1 comprising the amino acid sequence set forth in

SEQ ID NO: 30, HCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 43, and HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 32; and LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 40, LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 41, and LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 42; or

(2) a first VH + VL domain combination (specifically, a Fab arm) binding to c-Met, said first VH + VL domain combination having: HCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 36, HCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 38, and HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 39; and LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 40, LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 41, and LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 42, and

a second VH + VL domain combination (specifically, a Fab arm) binding to EGFR, said second VH+VL domain combination having: HCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 36, HCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 37, and HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 32; and LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 40, LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 41, and LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 42.

According to embodiments of the present invention, further, the antibody or antigen-binding fragment thereof comprises:

(1) a first VH + VL domain combination (specifically, a Fab arm) binding to c-Met, said first VH+VL domain combination having: a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 19, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 20, and

a second VH + VL domain combination (specifically, a Fab arm) binding to EGFR, said second VH + VL domain combination having: a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 15, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 20; or

(2) a first VH + VL domain combination (specifically, a Fab arm) binding to c-Met, said first VH+VL domain combination having: a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 19, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 20, and

a second VH + VL domain combination (specifically, a Fab arm) binding to EGFR, said second VH+VL domain combination having: a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 17, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 20.

In this case, the antibody or antigen-binding fragment thereof further comprises heavy chain constant regions and/or light chain constant regions. For example, the heavy chain constant region of the antibody comprises the amino acid sequence set forth in SEQ ID NO: 21; and the light chain constant region of the antibody comprises the amino acid sequence set forth in SEQ ID NO: 22. Alternatively, the heavy chain constant region of the antibody comprises the amino acid sequence set forth in SEQ ID NO: 23 (hIgG1; Knob) or SEQ ID NO: 24 (hIgG1; Hole); and the light chain constant region of the antibody comprises the amino acid sequence set forth in SEQ ID NO: 22.

Further, the antibody is a bispecific antibody, which has two different heavy chains but comprises two identical light chains. According to embodiments of the present invention, the bispecific antibody may comprise the amino acid sequence set forth in SEQ ID NO: 29 as a light chain.

According to embodiments of the present invention, the bispecific antibodies provided in the present invention are shown in Table 15 in the "Detailed Description". The bispecific antibodies shown in Table 15 comprise the following domains:

the bispecific antibody H15H1YCHV2xH22A12-2HV3-hIgG1, comprising two Fab arms respectively directed against c-Met and EGFR, with one Fab arm comprising a heavy chain variable region (SEQ ID NO: 19) and a light chain variable region (SEQ ID NO: 20) against c-Met, and the other Fab arm comprising a heavy chain variable region (SEQ ID NO: 15) and a light chain variable region (SEQ ID NO: 20) against EGFR; this bispecific antibody comprises two identical light chain variable regions (SEQ ID NO: 20) and corresponding two identical light chains (SEQ ID NO: 29);

the bispecific antibody H15H1YCHV2xH103G9-3HV2-hIgG1, comprising two Fab arms respectively directed against c-Met and EGFR, with one Fab arm comprising a heavy chain variable region (SEQ ID NO: 19) and a light chain variable region (SEQ ID NO: 20) against c-Met, and the other Fab arm comprising a heavy chain variable region (SEQ ID NO: 17) and a light chain variable region (SEQ ID NO: 20) against EGFR; this bispecific antibody comprises two identical light chain variable regions (SEQ ID NO: 20) and corresponding two identical light chains (SEQ ID NO: 29).

Further, the bispecific antibodies shown in Table 15, each as an IgG-like antibody, each comprise two different heavy chains (a heavy chain 1 and a heavy chain 2) and two identical light chains:

the bispecific antibody H15H1YCHV2xH22A12-2HV3-hIgG1, comprising the amino acid sequence set forth in SEQ ID NO: 25 as the heavy chain 1, comprising the amino acid sequence set forth in SEQ ID NO: 26 as the heavy chain 2, and comprising the amino acid sequence set forth in SEQ ID NO: 29 as the identical light chains;

the bispecific antibody H15H1YCHV2xH103G9-3HV2-hIgG1, comprising the amino acid sequence set forth in SEQ ID NO: 27 as the heavy chain 1, comprising the amino acid sequence set forth in SEQ ID NO: 28 as the heavy chain 2, and comprising the amino acid sequence set forth in SEQ ID NO: 29 as the identical light chains.

In a fifth aspect, the present invention provides a composition comprising the polypeptide of the first aspect, the nucleic acid molecule of the second aspect, or the antibody or antigen-binding fragment thereof of the fourth aspect. Said composition is preferably a pharmaceutical composition, which may optionally comprise pharmaceutically acceptable adjuvants, carriers or excipients. The pharmaceutical composition can be formulated into various dosage forms known in the medical or pharmaceutical field and administered by suitable routes.

In a sixth aspect, the present invention provides use of the polypeptide of the first aspect, the nucleic acid molecule of the second aspect, the antibody or antigen-binding fragment thereof of the fourth aspect, or the pharmaceutical composition of the fifth aspect, in the manufacture of a medicament.

The medicament may be an antibody-based drug used for treating diseases associated with

expression of a target protein or antigen thereof, for example, EGFR and/or c-Met expression-associated diseases. For example, the medicament is used for treating a tumor or cancer, such as lung cancer (e.g., non-small cell lung cancer, or small cell lung cancer), liver cancer, pancreatic cancer, gastric cancer, colorectal cancer, thyroid cancer, glioblastoma, head and neck cancer, breast cancer, esophageal cancer, cholangiocarcinoma, nasopharyngeal carcinoma, or melanoma.

In a seventh aspect, the present invention provides a method of treating a disease, comprising administering to a subject in need thereof the polypeptide of the first aspect, the nucleic acid molecule of the second aspect, the antibody or antigen-binding fragment thereof of the fourth aspect, or the pharmaceutical composition of the fifth aspect.

The method provided in the present invention is used for treating a tumor or cancer. Preferably, the tumor or cancer is lung cancer (e.g., non-small cell lung cancer, or small cell lung cancer), liver cancer, pancreatic cancer, gastric cancer, colorectal cancer, thyroid cancer, glioblastoma, head and neck cancer, breast cancer, esophageal cancer, cholangiocarcinoma, nasopharyngeal carcinoma, or melanoma. The subject may be a mammal, preferably a primate or rodent, more preferably a human.

In an eighth aspect, the present invention further provides an antibody or antigen-binding fragment thereof binding to EGFR, comprising the following heavy chain CDRs (HCDR1, HCDR2 and HCDR3) and light chain CDRs (LCDR1, LCDR2 and LCDR3):

(1) HCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 30, HCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 31, and HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 32; and LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 33, LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 34, and LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 35;

(2) HCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 36, HCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 37, and HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 32; and LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 33, LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 34, and LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 35;

(3) HCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 30, HCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 43, and HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 32; and LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 33, LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 34, and LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 35; or

(4) HCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 36, HCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 37, and HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 32; and LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 33, LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 34, and LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 35.

Preferably, the antibody or antigen-binding fragment thereof binding to EGFR comprises a heavy chain variable region and a light chain variable region, respectively comprising:

(1) the amino acid sequence set forth in SEQ ID NO: 10, and the amino acid sequence set forth in SEQ ID NO: 11;

(2) the amino acid sequence set forth in SEQ ID NO: 12, and the amino acid sequence set forth in SEQ ID NO: 11;

(3) the amino acid sequence set forth in SEQ ID NO: 15, and the amino acid sequence set forth in SEQ ID NO: 16; or

(4) the amino acid sequence set forth in SEQ ID NO: 17, and the amino acid sequence set forth in SEQ ID NO: 18.

The antibody or antigen-binding fragment thereof may be a murine antibody, a chimeric antibody or a humanized antibody; and may further comprise a heavy chain constant region and a light chain constant region. According to embodiments of the present invention, the heavy chain constant region may be a heavy chain constant region of an IgG1 or IgG4 antibody; the light chain constant region may be a light chain constant region of a kappa or lambda light chain. For example, the heavy chain constant region of the antibody comprises the amino acid sequence set forth in SEQ ID NO: 21 (hIgG1); and the light chain constant region of the antibody comprises the amino acid sequence set forth in SEQ ID NO: 22 (hkappa). Alternatively, the heavy chain

constant region of the antibody comprises the amino acid sequence set forth in SEQ ID NO: 23 (hIgG1; Knob) or SEQ ID NO: 24 (hIgG1; Hole); and the light chain constant region of the antibody comprises the amino acid sequence set forth in SEQ ID NO: 22.

In a ninth aspect, the present invention further provides an antibody or antigen-binding fragment thereof binding to c-Met, comprising the following heavy chain CDRs (HCDR1, HCDR2 and HCDR3) and light chain CDRs (LCDR1, LCDR2 and LCDR3):

HCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 36, HCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 38, and HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 39; and LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 40, LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 41, and LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 42.

Preferably, the antibody or antigen-binding fragment thereof binding to c-Met comprises a heavy chain variable region and a light chain variable region, respectively comprising:

(1) the amino acid sequence set forth in SEQ ID NO: 13, and the amino acid sequence set forth in SEQ ID NO: 14; or

(2) the amino acid sequence set forth in SEQ ID NO: 19, and the amino acid sequence set forth in SEQ ID NO: 20.

The antibody or antigen-binding fragment thereof may be a murine antibody, a chimeric antibody or a humanized antibody; and may further comprise a heavy chain constant region and a light chain constant region. According to embodiments of the present invention, the heavy chain constant region may be a heavy chain constant region of an IgG1 or IgG4 antibody; and the light chain constant region may be a light chain constant region of a kappa or lambda light chain. For example, the heavy chain constant region of the antibody comprises the amino acid sequence set forth in SEQ ID NO: 21 (hIgG1); and the light chain constant region of the antibody comprises the amino acid sequence set forth in SEQ ID NO: 22 (hkappa). Alternatively, the heavy chain constant region of the antibody comprises the amino acid sequence set forth in SEQ ID NO: 23 (hIgG1; Knob) or SEQ ID NO: 24 (hIgG1; Hole); and the light chain constant region of the antibody comprises the amino acid sequence set forth in SEQ ID NO: 22.

In a tenth aspect, the present invention provides use of the antibody or antigen-binding fragment thereof binding to EGFR of the eighth aspect or the antibody or antigen-binding fragment thereof binding to c-Met of the ninth aspect in the manufacture of a medicament.

The medicament may be an antibody-based drug used for treating diseases associated with expression of a target protein or antigen thereof, for example, EGFR and/or c-Met expression-associated diseases. For example, the medicament is used for treating a tumor or cancer, such as lung cancer (e.g., non-small cell lung cancer, or small cell lung cancer), liver cancer, pancreatic cancer, gastric cancer, colorectal cancer, thyroid cancer, glioblastoma, head and neck cancer, breast cancer, esophageal cancer, cholangiocarcinoma, nasopharyngeal carcinoma, or melanoma.

Compared with the prior art, the present invention provides a polypeptide derived from the light chain variable region or light chain of a specific humanized anti-c-Met antibody. Said polypeptide can serve as a common light chain for a bispecific antibody, and can be paired with heavy chain variable regions or heavy chains of an antibody with binding affinity and/or specificity for another target protein (e.g., EGFR), to construct a bispecific antibody. Experiments have demonstrated that said bispecific antibody maintains, or even exhibits, stronger binding affinities and/or specificities for two different target proteins. Consequentially, the polypeptides provided in the present invention have potential for use as a common light chain variable region or light chain for constructing bispecific antibodies. Additionally, the present invention also provides antibodies comprising said polypeptide as a common light chain variable region or light chain, for example, antibodies against EGFR and/or c-Met.

Brief Description of Drawings

Hereinafter, the embodiments of the present invention are described in detail with reference to the accompanying drawings, in which:

Figure 1 shows the binding activity of murine anti-EGFR antibodies and control antibodies to EGFR-overexpressing cells;

Figure 2 shows the blocking activity of murine anti-EGFR antibodies and control antibodies on EGFR binding to EGF;

Figure 3 shows the blocking activity of murine anti-EGFR antibodies and control antibodies on EGFR signaling;

Figure 4 shows the binding activity of humanized anti-EGFR antibodies and control antibodies to EGFR-overexpressing cells;

Figure 5 shows the blocking activity of humanized anti-EGFR antibodies and control antibodies on EGFR binding to EGF;

Figure 6 shows the blocking activity of humanized anti-EGFR antibodies and control antibodies on EGFR signaling;

Figure 7 shows the binding activity of murine anti-c-Met antibodies and control antibodies to c-Met-expressing cells;

Figure 8 shows the blocking activity of murine anti-c-Met antibodies and control antibodies on c-Met binding to HGF;

Figure 9 shows the blocking activity of murine anti-c-Met antibodies and control antibodies on c-Met signaling;

Figure 10 shows the binding activity of humanized anti-c-Met antibodies and control antibodies to c-Met-expressing cells;

Figure 11 shows the blocking activity of humanized anti-c-Met antibodies and control antibodies on c-Met binding to HGF;

Figure 12 shows the blocking activity of humanized anti-c-Met antibodies and control antibodies on c-Met signaling;

Figure 13 shows the binding activity of humanized anti-EGFR antibodies, EGFR × c-Met bispecific antibodies and control antibodies to EGFR-overexpressing cells;

Figure 14 shows the binding activity of humanized anti-c-Met antibodies, EGFR × c-Met bispecific antibodies and control antibodies to c-Met-overexpressing cells;

Figure 15 shows the blocking activity of EGFR × c-Met bispecific antibodies and control

antibodies on EGFR binding to EGF;

Figure 16 shows the blocking activity of EGFR × c-Met bispecific antibodies and control antibodies on c-Met binding to HGF;

Figure 17 shows the blocking activity of EGFR × c-Met bispecific antibodies and control antibodies on EGFR and c-Met signaling;

Figure 18 shows the inhibitory activity of EGFR × c-Met bispecific antibodies and control antibodies on tumor growth.

Detailed Description

The present invention is described below with reference to specific Examples. Those skilled in the art will appreciate that these Examples are only for illustrating the present invention and are not intended to limit the scope of the present invention in any way.

The experimental methods in the following Examples, unless otherwise specified, are conventional methods; the materials used in the following Examples, unless otherwise specified, are purchased from routine biochemical reagent commercial suppliers.

In constructing antibodies, the following constant region sequences were used in the following Examples:

> Human heavy chain CH1 and FC sequence (SEQ ID NO: 21):

hIgG1-WT:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

> Human light chain CL sequence (SEQ ID NO: 22):

hkappa:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

> Human heavy chain Knob CH1 and FC sequence (hIgG1; SEQ ID NO: 23):

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCR
DELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

> Human heavy chain Hole CH1 and FC sequence (hIgG1; SEQ ID NO: 24):

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSR
DELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

The following control antibodies were used in the following Examples:

Cetuximab: prepared by the applicant, Cat #: KA-1398-01;

MCLA129-METV2-hIgG1: prepared by the applicant, Cat #: KB-1214; constructed using the anti-c-Met variable region sequence from the known EGFR × c-Met bispecific antibody MCLA-129, together with the human light chain CL sequence (SEQ ID NO: 22) and the human heavy chain Knob CH1 and FC sequence (SEQ ID NO: 23);

JNJ372-EGFR×c-Met: prepared by the applicant, Cat #: KB-1123; constructed using the variable region sequences of the known EGFR × c-Met bispecific antibody Amivantamab (JNJ-372), using the KIH + crossmab bispecific antibody assembly technology;

MCLA129V1: prepared by the applicant, Cat #: KB-1217; i.e., the known EGFR × c-Met bispecific antibody MCLA-129;

AZD9592-hIgG1: prepared by the applicant, Cat #: KB-1380; the antibody moiety of AstraZeneca's AZD9592 (EGFR/c-Met ADC).

Example 1 Screening of a common light chain

After analysis of the prior art, it was found that the germlines of KV4 class have a higher expression frequency in mice and pair with mouse heavy chains at a higher frequency. Therefore, germline sequences of this class were selected, and from them the following mouse germline sequence KV4-59 was selected, which is relatively easy to humanize, and has good solubility, low immunogenicity, and no potential modification sites.

> KV4-59 (SEQ ID NO: 1):

QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMHWYQQKSGTSPKRWIYDTSKLASGVPA
RFSGSGSGTSYSLTISSMEAEDAATYYC**QQWSSNPP**FGSGTKLEIK

The above sequence was modified. Since CDR3 of the light chain (underlined; determined according to the KABAT definition scheme) plays an important role in the binding affinity of the antibody to the antigen, amino acid residues were added after CDR3 to improve affinity to potential antigens. The following three sequences were obtained:

> KV4-59-1 (SEQ ID NO: 2):

QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMHWYQQKSGTSPKRWIYDTSKLASGVPA
RFSGSGSGTSYSLTISSMEAEDAATYYC**QQWSSNPP*YT***FGSGTKLEIK

> KV4-59-2 (SEQ ID NO: 3):

QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMHWYQQKSGTSPKRWIYDTSKLASGVPA
RFSGSGSGTSYSLTISSMEAEDAATYYC**QQWSSNPP*WT***FGSGTKLEIK

> KV4-59-3 (SEQ ID NO: 4):

QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMHWYQQKSGTSPKRWIYDTSKLASGVPA

RFSGSGSGTSYSLTISSMEAEDAATYYC**QQWSSNPP*FT***FGSGTKLEIK

Chimeric antibodies were constructed by using the above three sequences as light chain variable region sequences of the antibodies respectively, together with the following heavy chain variable region sequence of a murine anti-chicken egg lysozyme antibody and human heavy and light chain constant regions.

> Heavy chain variable region sequence of the murine anti-chicken egg lysozyme antibody (SEQ ID NO: 5):

LDVKLQESGPGLVKPSQSLSLTCSVTGYSFTSGYYWNWIRLFPGNKLEWMGYVSYFGTN NYNPSLKNRFSITRDTSKNQFFLKLNSVSTEDTATYYCARGEEWDWSFDVWGTGTTVTV SS

By testing the properties of the three chimeric antibodies such as the antigen-binding affinity, expression level, thermal stability, solubility, freeze-thaw stability and the like, the inventors confirmed that when KV4-59-1 (SEQ ID NO: 2) was used as the light chain variable region sequence of the antibodies, the constructed antibodies have better properties.

Example 2 Construction of transgenic mice

Based on the lambda light chain gene sequences of the mouse antibodies, sgRNAs were designed, and a Cas9-gRNA target efficiency detection kit (Beijing ViewSolid Biotechnology Co., Ltd, Cat #: VK007-30T) was used for in vitro validation. The sgRNA sequences with high cleavage efficiency were selected: TGAATGCCATGTACTTATGG (SEQ ID NO: 6); AAGTTCAGCTCCTAAAATGG (SEQ ID NO: 7). The sgRNA sequences were synthesized and microinjected together with spCas91.1 protein into mouse fertilized oocytes. The sgRNA guides the Cas9 protein to cleave both ends of the fragment to be deleted of the target gene, causing DNA double-strand breaks. The genomic ends were repaired through non-homologous end joining (NHEJ), and the intervening target fragment was deleted, achieving knockout of the target gene. Two weeks after birth, genotyping was performed on the mice to confirm knockout of the lambda gene.

Based on the kappa light chain gene sequences of the mouse antibodies, sgRNAs were designed, and a Cas9-gRNA target efficiency detection kit (Beijing ViewSolid Biotechnology Co., Ltd, Cat

#: VK007-30T) was used for in vitro validation. The sgRNA sequences with high cleavage efficiency were selected: IGK-L1: GTGAATGCCATGTACTTATGG (SEQ ID NO: 8); IGK-R1: CAAGTTCAGCTCCTAAAATGG (SEQ ID NO: 9). The sgRNA sequences were synthesized and microinjected together with spCas91.1 protein into mouse fertilized oocytes. The sgRNA guides the Cas9 protein to cleave DNA at the target position to generate a nick, and knock-in of the DNA fragment was achieved at the target position through homologous recombination using a Donor DNA fragment comprising homology arms and the target fragment KV4-59-1 (SEQ ID NO: 2). Two weeks after birth, genotyping was performed on the mice to confirm knock-in of the common light chain gene. Heterozygous mice with lambda gene knockout and heterozygous mice with common light chain knock-in were selected for crossbreeding. Each offspring was genotyped two weeks after birth to detect common light chain knock-in and lambda knockout, until mice with common light chain knock-in at all alleles and knockout of both lambda and kappa light chains were obtained.

Example 3 Preparation of hybridoma cells secreting anti-EGFR antibodies

EGFR-mFc (KYINNO BIOTECHNOLOGY (BEIJING) CO., LTD. ("KYINNO"), Cat #: KP-1151) was used as an immunogen to immunize the mice constructed in Example 2. Mice with higher titers were selected and serum was collected therefrom; after dissection, spleens were taken and splenocytes were isolated. The splenocytes were fused with cultured myeloma cells to obtain hybridoma cells. The binding activity of hybridoma cell supernatants to the antigen protein EGFR-His (KYINNO, Cat #: KP-1150) was detected by ELISA, and multiple positive hybridoma cell lines secreting anti-EGFR antibodies were obtained.

Murine antibodies against EGFR were obtained from the positive hybridoma cell lines, with variable region sequences as follows (heavy and light chain CDRs are underlined, determined according to the KABAT definition scheme, *inf. id.*), wherein the murine antibodies 22A12-2 and 103G9-3 share a common light chain:

22A12-2 (the murine anti-EGFR antibody 1; also named "KD-22-0198")

> EGFR-22A12-2, VH (SEQ ID NO: 10; HCDR1/HCDR2/HCDR3: SEQ ID NO: 30/SEQ ID NO: 31/SEQ ID NO: 32):

EVQLQQSGPELVKPGASVKISCKASGYTFTDYYMNWVKQSHGKSLEWIGDINPNDGGT SYNQKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCAREILYYVTSFYFDYWGQGTTL TVSS

> EGFR-22A12-2, VL (SEQ ID NO: 11; LCDR1/LCDR2/LCDR3: SEQ ID NO: 33/SEQ ID NO: 34/SEQ ID NO: 35):

QIVLTQSPAIMSASPGEKVTMTCSATSSIGYMHWYQQKSGTSPKRWIYDTSKLASGVPAR FSGSGSGTFYSLTISSMEAEDAATYYCQLWSSDPPYTFGGGTKLEIK

103G9-3 (the murine anti-EGFR antibody 2; also named "KD-22-0220")

> EGFR-103G9-3, VH (SEQ ID NO: 12; HCDR1/HCDR2/HCDR3: SEQ ID NO: 36/SEQ ID NO: 37/SEQ ID NO: 32):

EVQLQQSGPELVKPGASVKISCKASGYTFTDYYINWVKQSHGKSLEWIGDINPNNDDTN YNQKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCAREILYYVTSFYFDYWGQGTTLT VSS

> EGFR-103G9-3, VL (SEQ ID NO: 11; LCDR1/LCDR2/LCDR3: SEQ ID NO: 33/SEQ ID NO: 34/SEQ ID NO: 35):

QIVLTQSPAIMSASPGEKVTMTCSATSSIGYMHWYQQKSGTSPKRWIYDTSKLASGVPAR FSGSGSGTFYSLTISSMEAEDAATYYCQLWSSDPPYTFGGGTKLEIK

Example 4 Binding activity of murine anti-EGFR antibodies to EGFR-overexpressing cells

The antibodies to be tested were serially diluted and incubated with CT26 cells overexpressing EGFR (KYINNO, Cat #: KC-1451), then incubated with the secondary antibody PE anti-mouse IgG Fc (Biolegend, Cat #: 405307) or the secondary antibody Goat anti-human IgG-PE (SouthernBiotech, Cat #: 2010-09). The antibody signal bound to cells was detected by flow cytometry. The results are set forth in Table 1 and Figure 1.

Table 1. Binding activity of murine anti-EGFR antibodies and control antibody to EGFR-overexpressing cells

Antibody	EC50 (μg/mL)
Cetuximab	0.01554
22A12-2	0.02418
103G9-3	0.02256

Example 5 Blocking activity of murine anti-EGFR antibodies on EGFR binding to EGF

The antibodies to be tested were serially diluted and incubated with 0.4 μg/ml of EGFR-mFc (KYINNO, Cat #: KP-1148) or EGFR-hFc (KYINNO, Cat #: KP-1149), then incubated in ELISA plates coated with EGF-His (2 μg/ml, Acro, Cat #: EGF-H52H3), and finally incubated with the secondary antibody HRP anti-mouse IgG (SIGMA, Cat #: A9309) or the secondary antibody HRP anti-human IgG (SIGMA, Cat #: A0170). The binding signal was detected by a microplate reader. The results are set forth in Table 2 and Figure 2.

Table 2. Blocking activity of murine anti-EGFR antibodies and control antibody on EGFR binding to EGF

Antibody	IC50 (μg/mL)
Cetuximab	0.4083
22A12-2	1.579
103G9-3	0.9592

Example 6 Blocking activity of murine anti-EGFR antibodies on EGFR signaling

The antibodies to be tested were serially diluted and incubated with 293T-NFAT-Luc2-EGFR (KYINNO, Cat #: KC-2952, which expresses luciferase upon EGFR signaling activation) for 30 minutes, then EGF-His (20 ng/ml, Acro, Cat #: EGF-H52H3) was added and incubated overnight. The activated luciferase signal was detected. The background activation is defined as the signal

detected in the absence of EGF activation, and the fold increase in activation intensity after EGF addition was plotted against antibody concentration to generate correlation curves. The results are set forth in Table 3 and Figure 3.

Table 3. Blocking activity of murine anti-EGFR antibodies and control antibody on EGFR signaling

Antibody	IC50 (μg/mL)
Cetuximab	0.3462
22A12-2	0.3735
103G9-3	0.2595

Example 7 Affinity of murine anti-EGFR antibodies to EGFR

ForteBio Octet was used to detect the affinity of antibodies to EGFR. The concentrations of mobile phase EGFR-His (KYINNO, Cat #: KP-1150) were 200, 100, 50, 25, 12.5, 6.25, 3.12 nM. The affinity results are set forth in Table 4.

Table 4. Affinity of murine anti-EGFR antibodies and control antibody to EGFR

Antibody	KD (M)	kon (1/Ms)	kdis (1/s)
Cetuximab	1.67E-09	1.09E+06	1.81E-03
22A12-2	1.15E-09	9.65E+05	1.11E-03
103G9-3	7.31E-10	9.89E+05	7.23E-04

Example 8 Construction of humanized anti-EGFR antibodies and chimeric anti-EGFR antibodies

The heavy and light chain variable regions of the two murine anti-EGFR antibodies were humanized to obtain humanized antibody sequences, with two humanized versions obtained for

the light chain variable region. The coding sequences of the humanized antibody sequences were respectively linked to the coding sequences of human IgG1 heavy chain constant region (SEQ ID NO: 21) and human kappa light chain constant region (SEQ ID NO: 22). The obtained coding genes were cloned into eukaryotic expression vectors, and expressed to obtain two humanized anti-EGFR antibodies, named H22A12-2V3-1-hIgG1 and H103G9-3V2-2-hIgG1 respectively, with heavy and light chain variable region sequences and full-length heavy and light chains as follows (heavy and light chain CDRs are underlined).

In addition, the coding sequences of the heavy and light chain variable regions of the two murine anti-EGFR antibodies were respectively linked to the coding sequences of human IgG1 heavy chain constant region (SEQ ID NO: 21) and human kappa light chain constant region (SEQ ID NO: 22). The obtained coding genes were cloned into eukaryotic expression vectors, and expressed to obtain two chimeric anti-EGFR antibodies.

H22A12-2V3-1-hIgG1 (the humanized anti-EGFR antibody 1; also named "KA-2029")

> EGFR-H22A12-2V3-1-hIgG1, VH (SEQ ID NO: 15; HCDR1/HCDR2/HCDR3: SEQ ID NO: 30/SEQ ID NO: 43/SEQ ID NO: 32):

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYMNWVRQAPGQGLEWIGDINPNDAG TSYNQKFKGRATLTVDKSTSTAYMELSSLRSEDTAVYYCAREILYYVTSFYFDYWGQGTT VTVSS

> EGFR-H22A12-2V3-1-hIgG1, VL (SEQ ID NO: 16; LCDR1/LCDR2/LCDR3: SEQ ID NO: 33/SEQ ID NO: 34/SEQ ID NO: 35):

EIVLTQSPDFQSVTPKEKVTITCSATSSIGYMHWYQQKPDTSPKRWIYDTSKLASGVPSRF SGSGSGTDYTLTINSLEAEDAATYYCQLWSSDPPYTFGQGTRLEIK

H103G9-3V2-2-hIgG1 (the humanized anti-EGFR antibody 2; also named "KA-2034")

> EGFR-H103G9-3V2-2-hIgG1, VH (SEQ ID NO: 17; HCDR1/HCDR2/HCDR3: SEQ ID NO: 36/SEQ ID NO: 37/SEQ ID NO: 32):

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYINWVRQAPGQGLEWIGDINPNNDDT NYNQKFKGRATLTVDKSTSTAYMELSSLRSEDTAVYYCAREILYYVTSFYFDYWGQGTT

VTVSS

> EGFR-H103G9-3V2-2-hIgG1, VL (SEQ ID NO: 18; LCDR1/LCDR2/LCDR3: SEQ ID NO: 33/SEQ ID NO: 34/SEQ ID NO: 35):

DIQLTQSPSTLSASVGDRVTITCSATSSIGYMHWYQQKPGTSPKRWIYDTSKLASGVPSRF
SGSGSGTEYTLTISSLQPDDFATYYCQLWSSDPPYTFGQGTRLEIK

Example 9 Binding activity of humanized anti-EGFR antibodies to EGFR-overexpressing cells

The antibodies to be tested were serially diluted and incubated with CT26 cells overexpressing EGFR (KYINNO, Cat #: KC-2063), then incubated with the secondary antibody Goat anti-human IgG-PE (SouthernBiotech, Cat #: 2010-09). The antibody signal bound to cells was detected by flow cytometry. The results are set forth in Table 5 and Figure 4.

Table 5. Binding activity of humanized anti-EGFR antibodies and control antibody to EGFR-overexpressing cells

Antibody	EC50 (μg/mL)	MFI.Max
Cetuximab	0.008277	53630
H22A12-2V3-1-hIgG1	0.01533	59797
H103G9-3V2-2-hIgG1	0.01116	58085

Example 10 Blocking activity of humanized anti-EGFR antibodies on EGFR binding to EGF

The antibodies to be tested were serially diluted and incubated with CT26 cells overexpressing EGFR (KYINNO, Cat #: KC-1451), then incubated with 0.2 μg/ml of EGF-mFc (Acro, Cat #: EGF-H525b), and finally incubated with FITC anti-mouse IgG2a (1:500, Biolegend, Cat #: 407106). The antibody signal bound to cells was detected by flow cytometry. The results are set forth in Table 6 and Figure 5.

Table 6. Blocking activity of humanized anti-EGFR antibodies and control antibody on EGFR binding to EGF

Antibody	IC50 (μg/mL)
Cetuximab	0.005269
H22A12-2V3-1-hIgG1	0.01106
H103G9-3V2-2-hIgG1	0.009781

Example 11 Blocking activity of humanized anti-EGFR antibodies on EGFR signaling

The antibodies to be tested were serially diluted and incubated with 293T-NFAT-Luc2-EGFR (KYINNO, Cat #: KC-2952, which expresses luciferase upon EGFR signaling activation) for 30 minutes, then EGF-His (20 ng/ml, Acro, Cat #: EGF-H52H3) was added and incubated overnight. The activated luciferase signal was detected. The background activation is defined as the signal detected in the absence of EGF activation, and the fold increase in activation intensity after EGF addition was plotted against antibody concentration to generate correlation curves. The results are set forth in Table 7 and Figure 6.

Table 7. Blocking activity of humanized anti-EGFR antibodies and control antibody on EGFR signaling

Antibody	IC50 (nM)
Cetuximab	0.2552
H22A12-2V3-1-hIgG1	0.3032
H103G9-3V2-2-hIgG1	0.2617

Example 12 Preparation of hybridoma cells secreting anti-c-Met antibodies

HGFR SEMA-mFc (KYINNO, Cat #: KP-1271) was used as an immunogen to immunize the mice constructed in Example 2. Mice with higher titers were selected and serum was collected therefrom; after dissection, spleens were taken and splenocytes were isolated. The splenocytes

were fused with cultured myeloma cells to obtain hybridoma cells. The binding activity of hybridoma cell supernatants to the antigen protein was detected by ELISA, and multiple positive hybridoma cell lines secreting anti-c-Met antibodies were obtained.

Murine antibodies against c-Met were obtained from the positive hybridoma cell lines, with variable region sequences as follows (heavy and light chain CDRs are underlined, determined according to the KABAT definition scheme, *inf. id.*):

15H1Y1C (the murine anti-c-Met antibody, also named "KD-23-0480")

> MET-15H1Y1C, VH (SEQ ID NO: 13; HCDR1/HCDR2/HCDR3: SEQ ID NO: 36/SEQ ID NO: 38/SEQ ID NO: 39):

EVQLQQSGPELVKPGTSVKISCKASGYTFTDYYINWVKQSHGKSLEWIGDINPNNGGTDYNQKFKDKATLTVDKSSSTAYMELRSLTSEDSAVYFCASDLNGNYEVDYWGQGTSVTVSS

> MET-15H1Y1C, VL (SEQ ID NO: 14; LCDR1/LCDR2/LCDR3: SEQ ID NO: 40/SEQ ID NO: 41/SEQ ID NO: 42):

QIVLTQSPAIMSASPGEKVTMTCSASSSVTFMHWYQQKSGTSPKRWIYDTSNLASGVPARFSGSGSGTSYSLTISSMEAEDAATYYCQQWGDDPPYTFGGGTKLEIK

Example 13 Binding activity of the murine anti-c-Met antibody to c-Met-expressing cells

The antibodies to be tested were serially diluted and incubated with human gastric cancer MKN45 cells expressing c-Met (KYINNO, Cat #: KC-0412), then incubated with the secondary antibody PE anti-mouse IgG Fc (Biolegend, Cat #: 405307) or the secondary antibody Goat anti-human IgG-PE (SouthernBiotech, Cat #: 2010-09). The antibody signal bound to cells was detected by flow cytometry. The results are set forth in Table 8 and Figure 7.

Table 8. Binding activity of the murine anti-c-Met antibody and control antibody to c-Met-expressing cells

Antibody	EC50 (μg/mL)
MCLA129-METV2-hIgG1	0.04975
15H1Y1C	0.02612

Example 14 Blocking activity of the murine anti-c-Met antibody on c-Met binding to HGF

The antibodies to be tested were serially diluted and incubated with human gastric cancer MKN45 cells expressing c-Met (KYINNO, Cat #: KC-0412), then incubated with 0.04 μg/ml of HGF-His (Acro, Cat #: HGF-H52H3), and finally incubated with the anti-His antibody APC anti-His Tag (1:500, Biolegend, Cat #: 362605). The antibody signal bound to cells was detected by flow cytometry. The results are set forth in Table 9 and Figure 8.

Table 9. Blocking activity of the murine anti-c-Met antibody and control antibody on c-Met binding to HGF

Antibody	IC50 (μg/mL)
MCLA129-METV2-hIgG1	0.1345
15H1Y1C	0.05016

Example 15 Blocking activity of the murine anti-c-Met antibody on c-Met signaling

The antibodies to be tested were serially diluted and incubated with 293T-SRE-Luc2-MET (KYINNO, Cat #: KC-2056, which expresses luciferase upon c-Met signaling activation) for 30 minutes, then RH-HGF (1 ng/ml, Acro, Cat #: HGF-H5218) was added and incubated overnight. The activated luciferase signal was detected. The background activation is defined as the signal detected in the absence of RH-HGF activation, and the fold increase in activation intensity after RH-HGF addition was plotted against antibody concentration to generate correlation curves. The results are set forth in Table 10 and Figure 9.

Table 10. Blocking activity of the murine anti-c-Met antibody and control antibody on c-Met

signaling

Antibody	IC50 (μg/mL)
MCLA129-METV2-hIgG1	0.042
15H1Y1C	0.012

Example 16 Construction of humanized anti-c-Met antibody and chimeric anti-c-Met antibody

The heavy and light chain variable regions of the murine anti-c-Met antibody were humanized to obtain humanized antibody sequences. The coding sequences of the humanized antibody sequences were respectively linked to the coding sequences of human IgG1 heavy chain constant region (SEQ ID NO: 21) and human kappa light chain constant region (SEQ ID NO: 22). The obtained coding genes were cloned into eukaryotic expression vectors, and expressed to obtain an anti-c-Met humanized antibody, named H15H1Y1CV2-hIgG1, with heavy and light chain variable region sequences and full-length heavy and light chains as follows (heavy and light chain CDRs are underlined).

In addition, the coding sequences of the heavy and light chain variable regions of the murine anti-c-Met antibody were respectively linked to the coding sequences of human IgG1 heavy chain constant region (SEQ ID NO: 21) and human kappa light chain constant region (SEQ ID NO: 22). The obtained coding genes were cloned into eukaryotic expression vectors, and expressed to obtain a chimeric anti-c-Met antibody.

H15H1Y1CV2-hIgG1 (the humanized anti-c-Met antibody; also named "KA-2427")

> MET-H15H1Y1CV2-hIgG1, VH (SEQ ID NO: 19; HCDR1/HCDR2/HCDR3: SEQ ID NO: 36/SEQ ID NO: 38/SEQ ID NO: 39)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYINWVRQAPGKGLEWIGDINPNNGGTDYNQKFKDRATLTVDKSTSTAYMELRSLRSEDTAVYFCASDLNGNYEVDYWGQGTTVTVSS

> MET-H15H1Y1CV2-hIgG1, VL (SEQ ID NO: 20; LCDR1/LCDR2/LCDR3: SEQ ID NO:

40/SEQ ID NO: 41/SEQ ID NO: 42)

DIQLTQSPSTLSASVGDRVTITCSASSSVTFMHWYQQKPGTSPKRWIYDTSNLASGVPSRF SGSGSGTEYTLTISSLQPDDFATYYCQQWGDDPPYTFGQGTRLEIK

Example 17 Binding activity of the humanized anti-c-Met antibody to c-Met-expressing cells

The antibodies to be tested were serially diluted and incubated with human gastric cancer MKN45 cells expressing c-Met (KYINNO, Cat #: KC-0412), then incubated with the secondary antibody Goat anti-human IgG-PE (SouthernBiotech, Cat #: 2010-09). The antibody signal bound to cells was detected by flow cytometry. The results are set forth in Table 11 and Figure 10.

Table 11. Binding activity of the humanized anti-c-Met antibody and control antibody to c-Met-expressing cells

Antibody	EC50 (μg/mL)
MCLA129-METV2-hIgG1	0.06661
H15H1Y1CV2-hIgG1	0.01492

Example 18 Blocking activity of the humanized anti-c-Met antibody on c-Met binding to HGF

The antibodies to be tested were serially diluted and incubated with human gastric cancer MKN45 cells expressing c-Met (KYINNO, Cat #: KC-0412), then incubated with 0.04 μg/ml of HGF-His (Acro, Cat #: HGF-H52H3), and finally incubated with the anti-His antibody APC anti-His Tag (1:500, Biolegend, Cat #: 362605). The antibody signal bound to cells was detected by flow cytometry. The results are set forth in Table 12 and Figure 11.

Table 12. Blocking activity of the humanized anti-c-Met antibody and control antibody on c-Met binding to HGF

Antibody	IC50 (μg/mL)

MCLA129-METV2-hIgG1	0.2104
H15H1Y1CV2-hIgG1	0.02541

Example 19 Blocking activity of the humanized anti-c-Met antibody on c-Met signaling

The antibodies to be tested were serially diluted and incubated with 293T-SRE-Luc2-MET (KYINNO, Cat #: KC-2056, which expresses luciferase upon c-Met signaling activation) for 30 minutes, then RH-HGF (1 ng/ml, Acro, Cat #: HGF-H5218) was added and incubated overnight. The activated luciferase signal was detected. The background activation is defined as the signal detected in the absence of RH-HGF activation, and the fold increase in activation intensity after RH-HGF addition was plotted against antibody concentration to generate correlation curves. The results are set forth in Table 13 and Figure 12.

Table 13. Blocking activity of the humanized anti-c-Met antibody and control antibody on c-Met signaling

Antibody	IC50 (μg/mL)
MCLA129-METV2-hIgG1	0.021
H15H1Y1CV2-hIgG1	0.007

Example 20 Affinity of the humanized anti-c-Met antibody to c-Met (HGFR)

ForteBio Octet was used to detect the affinity of antibodies to c-Met. The concentrations of mobile phase human c-Met-His (KYINNO, Cat #: KP-1153) were 200, 100, 50, 25, 12.5, 6.25, 3.12 nM. The affinity results are set forth in Table 14.

Table 14. Affinity of the humanized anti-c-Met antibody and control antibody to c-Met

Antibody	KD (M)	kon (1/Ms)	kdis (1/s)

MCLA129-METV2-hIgG1	3.79E-09	1.89E+05	7.17E-04
H15H1Y1CV2-hIgG1	2.75E-10	5.75E+05	1.58E-04

Example 21 Construction of EGFR × c-Met bispecific antibodies

The coding sequence of the light chain variable region of the humanized anti-c-Met antibody H15H1Y1CV2-hIgG1 (SEQ ID NO: 20) was linked to the coding sequence of human kappa light chain constant region (SEQ ID NO: 22) to obtain the common light chain of the bispecific antibodies.

The coding sequence of the heavy chain variable region of the humanized anti-c-Met antibody H15H1Y1CV2-hIgG1 (SEQ ID NO: 19) was linked to the coding sequence of human heavy chain Knob CH1 and FC sequence (SEQ ID NO: 23); the coding sequence of the heavy chain variable region of the humanized anti-EGFR antibody 1, i.e., H22A12-2V3-1-hIgG1 (SEQ ID NO: 15), was linked to the coding sequence of human heavy chain Hole CH1 and FC sequence (SEQ ID NO: 24), or the coding sequence of the heavy chain variable region of the humanized anti-EGFR antibody 2, i.e., H103G9-3V2-2-hIgG1 (SEQ ID NO: 17), was linked to the coding sequence of human heavy chain Hole CH1 and FC sequence (SEQ ID NO: 24).

The obtained coding sequences were respectively cloned into eukaryotic expression vectors to obtain four recombinant expression plasmids (one plasmid encoding the common light chain, one plasmid encoding the heavy chain against c-Met, and two plasmids respectively encoding the heavy chains against EGFR). The HEK293F expression system was used for transient co-transfection of three plasmids (selecting one of the two plasmids encoding the heavy chains against EGFR) to produce EGFR × c-Met bispecific antibodies. Briefly, for a 1 L shake flask, HEK293F cells were seeded at a concentration of 1×10^6 cells/mL in 250 mL of medium, incubated at 110 rpm, 5% CO_2; the next day, the three prepared expression vectors were mixed with transfection reagent at a certain ratio, and the transfection complex was added to the cells at a cell density of 2×10^6 cells/mL; after 24 h, nutrients and DNA inhibitors were supplemented; after 5-7 days of cell culture, the expression supernatant was harvested, centrifuged, filtered, and purified using MabSelectSure affinity chromatography column (GE Healthcare). The purified

antibodies were assessed for purity by SDS-PAGE electrophoresis, and antibody concentration was measured with Nanodrop.

The domains and sequences thereof comprised in the obtained EGFR × c-Met bispecific antibodies are set forth in Table 15 and below (the italicized sequences in the following are constant regions).

Table 15. EGFR × c-Met bispecific antibodies comprising the common light chain

Bispecific antibody	Heavy chain against c-Met		Heavy chain against EGFR		Common light chain		
	Heavy chain variable region	Heavy chain constant region	Heavy chain variable region	Heavy chain constant region	Light chain variable region	Light chain constant region	Light chain
H15H1YCHV2×H22A12-2HV3-hIgG1	SEQ ID NO: 19	SEQ ID NO: 23【Knob】	SEQ ID NO: 15	SEQ ID NO: 24【Hole】	SEQ ID NO: 20	SEQ ID NO: 22	SEQ ID NO: 29
H15H1YCHV2×H103G9-3HV2-hIgG1	SEQ ID NO: 19	SEQ ID NO: 23【Knob】	SEQ ID NO: 17	SEQ ID NO: 24【Hole】	SEQ ID NO: 20	SEQ ID NO: 22	SEQ ID NO: 29

H15H1YCHV2xH22A12-2HV3-hIgG1 (the EGFR × c-Met bispecific antibody 1; also named "KA-2597")

> MET-H15H1Y1CV2-Knob (heavy chain 1, SEQ ID NO: 25; VH: SEQ ID NO: 19; CH[Knob]: SEQ ID NO: 23)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYINWVRQAPGKGLEWIGDINPNNGGT

DYNQKFKDRATLTVDKSTSTAYMELRSLRSEDTAVYFCASDLNGNYEVDYWGQGTTVT
VSS*ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL*
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLF
PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPG

> EGFR-H22A12-2V3-Hole (heavy chain 2, SEQ ID NO: 26; VH: SEQ ID NO: 15; CH[Hole]: SEQ ID NO: 24)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYMNWVRQAPGQGLEWIGDINPNDAG
TSYNQKFKGRATLTVDKSTSTAYMELSSLRSEDTAVYYCAREILYYVTSFYFDYWGQGTT
VTVSS*ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS*
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLS
CAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPG

> Common light chain (SEQ ID NO: 29; VL: SEQ ID NO: 20; LC: SEQ ID NO: 22)

DIQLTQSPSTLSASVGDRVTITCSASSSVTFMHWYQQKPGTSPKRWIYDTSNLASGVPSRF
SGSGSGTEYTLTISSLQPDDFATYYCQQWGDDPPYTFGQGTRLEIK*RTVAAPSVFIFPPSDE*
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE
KHKVYACEVTHQGLSSPVTKSFNRGEC

H15H1YCHV2×H103G9-3HV2-hIgG1 (the EGFR × c-Met bispecific antibody 2; also named "KA-2599")

> MET-H15H1Y1CV2-Knob (heavy chain 1, SEQ ID NO: 27; VH: SEQ ID NO: 19; CH[Knob]: SEQ ID NO: 23)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYINWVRQAPGKGLEWIGDINPNNGGT
DYNQKFKDRATLTVDKSTSTAYMELRSLRSEDTAVYFCASDLNGNYEVDYWGQGTTVT

VSS*ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPG*

> EGFR-H103G9-3HV2-Hole (heavy chain 2, SEQ ID NO: 28; VH: SEQ ID NO: 17; CH[Hole]: SEQ ID NO: 24)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYINWVRQAPGQGLEWIGDINPNNDDT NYNQKFKGRATLTVDKSTSTAYMELSSLRSEDTAVYYCAREILYYVTSFYFDYWGQGTT VTVSS*ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLS CAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPG*

> Common light chain (SEQ ID NO: 29; VL: SEQ ID NO: 20; LC: SEQ ID NO: 22)

DIQLTQSPSTLSASVGDRVTITCSASSSVTFMHWYQQKPGTSPKRWIYDTSNLASGVPSRF SGSGSGTEYTLTISSLQPDDFATYYCQQWGDDPPYTFGQGTRLEIK*RTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC*

Example 22 Binding activity of humanized anti-EGFR antibodies, the humanized anti-c-Met antibody, and EGFR × c-Met bispecific antibodies to EGFR-overexpressing and c-Met (HGFR)-overexpressing cells

CT26 cells overexpressing EGFR were constructed, named "CT26-EGFR cells" (KYINNO, Cat #: KC-2063). CHOK1 cells overexpressing c-Met were constructed, named "CHOK1-cMET cells" (KYINNO, Cat #: KC-2524).

The antibodies to be tested were serially diluted and incubated with CT26-EGFR cells or CHOK1-cMET cells, then incubated with the secondary antibody Goat anti-human IgG-PE (SouthernBiotech, Cat #: 2010-09). The antibody signal bound to cells was detected by flow cytometry. The results are set forth in Table 16 and Figure 13 (EGFR), and Table 17 and Figure 14 (c-Met).

Table 16. Binding activity of humanized anti-EGFR antibodies, EGFR × c-Met bispecific antibodies and control antibodies to EGFR-overexpressing cells

Antibody	EC50 (μg/mL)	MFI.Max
JNJ372-EGFR×c-Met	0.0672	46140
MCLA129V1	0.5458	37975
AZD9592-hIgG1	N/A	18728
H15H1YCHV2×H22A12-2HV3-hIgG1	0.06741	103802
H15H1YCHV2×H103G9-3HV2-hIgG1	0.0801	74245

Table 17. Binding activity of the humanized anti-c-Met antibody, EGFR × c-Met bispecific antibodies and control antibodies to c-Met-overexpressing cells

Antibody	EC50 (ug/mL)	MFI.Max
JNJ372-EGFR×c-Met	0.1222	13582
MCLA129V1	0.3421	14863
AZD9592-hIgG1	0.02142	10969
MCLA129-METV2-hIgG1	0.08725	7714

H15H1Y1CV2-hIgG1	0.006105	7335
H15H1YCHV2×H22A12-2HV3-hIgG1	0.01205	14598
H15H1YCHV2×H103G9-3HV2-hIgG1	0.01784	11731

Example 23 Affinity of EGFR × c-Met bispecific antibodies to human EGFR and c-Met (HGFR)

ForteBio Octet was used to detect the affinity of antibodies to EGFR and c-Met. The mobile phases used were human EGFR-His (KYINNO, Cat #: KP-1150) and human c-Met-His (KYINNO, Cat #: KP-1153), respectively, at concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.12 nM. The results are set forth in Table 18.

Table 18. Affinity of EGFR × c-Met bispecific antibodies and control antibodies to EGFR and c-Met

Antibody	Antigen	ka (1/Ms)	kd (1/s)	KD (M)
JNJ372-EGFR×cMet	Human HGFR (c-Met)	5.35E+05	2.93E-04	5.49E-10
MCLA129V1	Human HGFR (c-Met)	2.05E+05	2.53E-04	1.23E-09
AZD9592-hIgG1	Human HGFR (c-Met)	4.73E+05	8.80E-04	1.86E-09
H15H1YCHV2×H22A12-2HV3-hIgG1	Human HGFR (c-Met)	7.77E+05	1.63E-05	2.10E-11

H15H1YCHV2×H103G9-3HV2-hIgG1	Human HGFR (c-Met)	7.12E+05	1.69E-04	2.37E-10
JNJ372-EGFR×cMet	Human EGFR	3.87E+05	1.28E-03	3.31E-09
MCLA129V1	Human EGFR	3.96E+05	4.32E-03	1.09E-08
AZD9592-hIgG1	Human EGFR	1.43E+05	1.79E-03	1.26E-08
H15H1YCHV2×H22A12-2HV3-hIgG1	Human EGFR	1.04E+06	1.06E-03	1.02E-09
H15H1YCHV2×H103G9-3HV2-hIgG1	Human EGFR	1.61E+06	1.62E-03	1.01E-09

Example 24 Blocking activity of EGFR × c-Met bispecific antibodies on EGFR binding to EGF

The antibodies to be tested were serially diluted and incubated with CT26 cells overexpressing EGFR (KYINNO, Cat #: KC-1451), then incubated with 0.2 μg/ml of EGF-mFc (Acro, Cat #: EGF-H525b), and finally incubated with FITC anti-mouse IgG2a (1:500, Biolegend, Cat #: 407106). The antibody signal bound to cells was detected by flow cytometry. The results are set forth in Table 19 and Figure 15.

Table 19. Blocking activity of EGFR × c-Met bispecific antibodies and control antibodies on EGFR binding to EGF

Antibody	IC50 (μg/mL)
MCLA129V1	7.627

AZD9592-hIgG1	N/A
H15H1YCHV2×H22A12-2HV3-hIgG1	0.3545
H15H1YCHV2×H103G9-3HV2-hIgG1	1.137

Example 25 Blocking activity of EGFR × c-Met bispecific antibodies on c-Met binding to HGF

The antibodies to be tested were serially diluted and incubated with human gastric cancer MKN45 cells expressing c-Met (KYINNO, Cat #: KC-0412), then incubated with 0.04 μg/ml of HGF-His (Acro, Cat #: HGF-H52H3), and finally incubated with the anti-His antibody APC anti-His Tag (1:500, Biolegend Cat #: 362605). The antibody signal bound to cells was detected by flow cytometry. The results are set forth in Table 20 and Figure 16.

Table 20. Blocking activity of EGFR × c-Met bispecific antibodies and control antibodies on c-Met binding to HGF

Antibody	IC50 (μg/mL)
JNJ372-EGFR×cMet	0.0155
MCLA129V1	0.03721
AZD9592-hIgG1	0.0324
H15H1YCHV2×H22A12-2HV3-hIgG1	0.005727
H15H1YCHV2×H103G9-3HV2-hIgG1	0.01044

Example 26 Blocking activity of EGFR × c-Met bispecific antibodies on EGFR and c-Met signaling

The antibodies to be tested at a final concentration of 10 μg/ml were incubated with H1975 cells (KYINNO, Cat #: KC-1566), and simultaneously EGF-mFc (Acro, Cat #: EGF-H525b) at a final concentration of 50 ng/ml and HGF-His (Acro, Cat #: HGF-H52H3) at a final concentration of

100 ng/ml were added. After incubation at 37°C for 15 min, lysis buffer was added to each well to lyse the cells, and then the phosphorylation levels of EGFR, c-Met, Akt and Erk in the cells were measured by western blotting to analyze the activation of EGFR and c-Met signaling pathways, thereby evaluating the efficiency of antibodies in inhibiting EGFR and c-Met signaling pathways. The results are set forth in Figure 17.

The results showed that both EGFR × c-Met bispecific antibodies have significantly stronger inhibitory efficiency on EGFR and c-Met signaling pathways than the control antibodies.

Example 27 Inhibitory activity of EGFR × c-Met bispecific antibodies on tumor growth

The immunodeficient mice B-NDG (Biocytogen, Cat #: 110586) were subcutaneously inoculated with one million H1975 tumor cells overexpressing HGF (KYINNO, Cat #: KC-1566). Tumor size was measured three times per week, and tumor volume was calculated using the formula: 0.5×length×width×height. When tumors grew to ~100 mm^3, the tumor-bearing mice were randomly grouped and intravenously injected with the antibodies to be tested. Antibodies were injected twice weekly for 3 times, then changed to once weekly. The results are set forth in Figure 18.

The results showed that the EGFR × c-Met bispecific antibody KA-2597 significantly inhibited tumor growth, and the antitumor effect thereof was superior to that of the control antibodies.

The above description of embodiments of the present invention does not limit the present invention. Those skilled in the art can make various changes or modifications according to the present invention without departing from the spirit of the present invention, and all such changes or modifications shall fall within the scope of the appended claims of the present invention.

What is claimed is:

1. A polypeptide, the amino acid sequence of which comprises: the amino acid sequence set forth in SEQ ID NO: 40, the amino acid sequence set forth in SEQ ID NO: 41, and the amino acid sequence set forth in SEQ ID NO: 42.

2. The polypeptide according to claim 1, characterized in that the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 20;

preferably, the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 29.

3. A nucleic acid molecule comprising a nucleotide sequence encoding the polypeptide according to claim 1 or 2.

4. Use of the polypeptide according to claim 1 or 2, or the nucleic acid molecule according to claim 3, in the construction of an antibody.

5. An antibody or antigen-binding fragment thereof comprising the polypeptide according to claim 1 or 2;

preferably, the antibody or antigen-binding fragment thereof comprises the polypeptide according to claim 1 or 2 as a light chain variable region or light chain;

preferably, the antibody or antigen-binding fragment thereof is an IgG-like antibody or an antibody format such as scFv, dsFv, (dsFv)2, Fab, Fab', F(ab')2 or Fv.

6. The antibody or antigen-binding fragment thereof according to claim 5, characterized in that the antibody or antigen-binding fragment thereof comprises the following heavy chain CDRs (HCDR1, HCDR2 and HCDR3) and light chain CDRs (LCDR1, LCDR2 and LCDR3):

(1) HCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 36, HCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 38, and HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 39; and LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 40, LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 41, and LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 42;

(2) HCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 30, HCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 43, and HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 32; and LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 40, LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 41, and LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 42; or

(3) HCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 36, HCDR2

comprising the amino acid sequence set forth in SEQ ID NO: 37, and HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 32; and LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 40, LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 41, and LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 42.

7. The antibody or antigen-binding fragment thereof according to claim 5 or 6, characterized in that the antibody or antigen-binding fragment thereof comprises a heavy chain variable region and a light chain variable region respectively comprising:

(1) the amino acid sequence set forth in SEQ ID NO: 13, and the amino acid sequence set forth in SEQ ID NO: 14;

(2) the amino acid sequence set forth in SEQ ID NO: 19, and the amino acid sequence set forth in SEQ ID NO: 20;

(3) the amino acid sequence set forth in SEQ ID NO: 15, and the amino acid sequence set forth in SEQ ID NO: 20; or

(4) the amino acid sequence set forth in SEQ ID NO: 17, and the amino acid sequence set forth in SEQ ID NO: 20.

8. The antibody or antigen-binding fragment thereof according to any one of claims 5 to 7, characterized in that the antibody or antigen-binding fragment thereof has at least two VH + VL domain combinations, which are identical, thereby binding to the same target protein; or are different, thereby binding to different target proteins;

preferably, the two VH + VL domain combinations have a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 20.

9. The antibody or antigen-binding fragment thereof according to any one of claims 5 to 8, characterized in that the antibody or antigen-binding fragment thereof comprises:

(1) a first VH + VL domain combination binding to c-Met, having: HCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 36, HCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 38, and HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 39; and LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 40, LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 41, and LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 42, and

a second VH + VL domain combination binding to EGFR, having: HCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 30, HCDR2 comprising the amino acid sequence

set forth in SEQ ID NO: 43, and HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 32; and LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 40, LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 41, and LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 42; or

(2) a first VH + VL domain combination binding to c-Met, having: HCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 36, HCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 38, and HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 39; and LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 40, LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 41, and LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 42, and

a second VH + VL domain combination binding to EGFR, having: HCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 36, HCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 37, and HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 32; and LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 40, LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 41, and LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 42.

10. The antibody or antigen-binding fragment thereof according to any one of claims 5 to 9, characterized in that the antibody or antigen-binding fragment thereof comprises:

(1) a first VH + VL domain combination binding to c-Met, having: a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 19, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 20, and

a second VH + VL domain combination binding to EGFR, having: a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 15, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 20; or

(2) a first VH + VL domain combination binding to c-Met, having: a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 19, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 20, and

a second VH + VL domain combination binding to EGFR, having: a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 17, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 20.

11. The antibody or antigen-binding fragment thereof according to any one of claims 5 to 10,

characterized in that the antibody or antigen-binding fragment thereof further comprises a heavy chain constant region and/or a light chain constant region;

preferably, the antibody is a bispecific antibody.

12. A composition comprising the polypeptide according to claim 1 or 2, the nucleic acid molecule according to claim 3, or the antibody or antigen-binding fragment thereof according to any one of claims 5 to 11.

13. Use of the polypeptide according to claim 1 or 2, the nucleic acid molecule according to claim 3, the antibody or antigen-binding fragment thereof according to any one of claims 5 to 11, or the composition according to claim 12, in the manufacture of a medicament.

14. An antibody or antigen-binding fragment thereof against EGFR, said antibody or antigen-binding fragment thereof comprising the following heavy chain CDRs (HCDR1, HCDR2 and HCDR3) and light chain CDRs (LCDR1, LCDR2 and LCDR3):

(1) HCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 30, HCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 31, and HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 32; and LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 33, LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 34, and LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 35;

(2) HCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 36, HCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 37, and HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 32; and LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 33, LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 34, and LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 35;

(3) HCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 30, HCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 43, and HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 32; and LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 33, LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 34, and LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 35; or

(4) HCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 36, HCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 37, and HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 32; and LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 33, LCDR2 comprising the amino acid sequence set forth in

SEQ ID NO: 34, and LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 35.

15. An antibody or antigen-binding fragment thereof against c-Met, said antibody or antigen-binding fragment thereof comprising the following heavy chain CDRs (HCDR1, HCDR2 and HCDR3) and light chain CDRs (LCDR1, LCDR2 and LCDR3):

HCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 36, HCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 38, and HCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 39; and LCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 40, LCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 41, and LCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 42.