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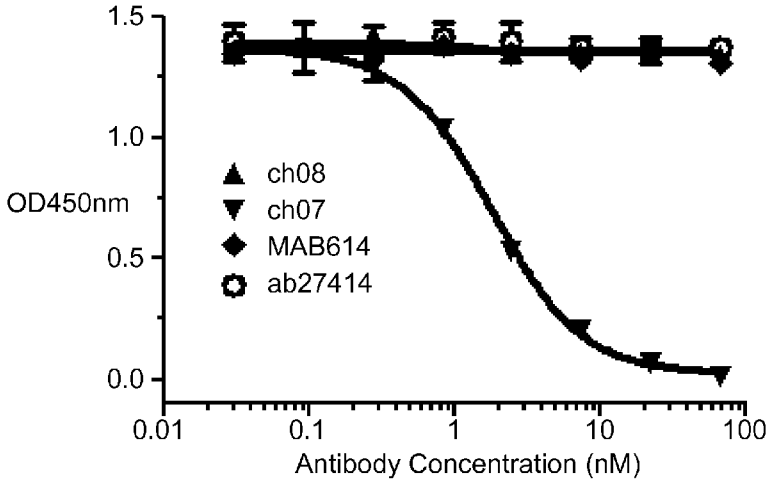
(54) Title: ANTI-IL-13 RECEPTOR ALPHA 2 ANTIBODIES AND ANTIBODY-DRUG CONJUGATES

(57) Abstract: Disclosed herein are anti-IL-13-Rα2 antibodies and antibody drug conjugates and methods for preparing and using the same.

FIG. 2A



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— *as to the identity of the inventor (Rule 4.17(i))*

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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ANTI-IL-13 RECEPTOR ALPHA 2 ANTIBODIES AND ANTIBODY-DRUG CONJUGATES

Field of the Invention

This invention pertains to anti-IL-13 receptor alpha 2 (IL-13-Rα2) antibodies and antibody-drug conjugates for the treatment of cancer.

Sequence Listing

The instant application contains a Sequence Listing which has been submitted via EFS and is hereby incorporated by reference in its entirety.

Background of the Invention

High levels of IL-13-Rα2 have been identified in a number of tumor cells, including pancreatic, breast, ovarian and malignant gliomas. In contrast, only a few types of normal tissues express IL-13-Rα2, and only at low levels. The treatment of cancer has improved over the past decade with surgery, radiation therapy, and chemotherapy as the primary treatment options. Such treatments can extend survival and/or relieve symptoms in many patients but are not likely to produce a cure for many patients. There remains a significant need for additional therapeutic options for cancers.

Therefore, anti-IL-13-Rα2 antibody-drug conjugates that can exert a clinically useful cytotoxic effect on IL-13-Rα2 expressing tumor cells, particularly without exerting undesirable effects on non-IL-13-Rα2 expressing cells, fulfill an unmet clinical need in the treatment of various IL-13-Rα2 expressing tumor cells.

Summary of the Invention

The present invention provides anti-IL-13-Rα2 antibody-drug conjugates and methods of use for the treatment of cancer.

The present invention provides an isolated antibody or antigen-binding fragment that specifically binds to human IL-13-Rα2 wherein the antibody comprises: a heavy chain variable region comprising a CDR1, CDR2, and CDR3 of the VH sequence of

SEQ ID NO: 1 and, a light chain variable region comprising a CDR1, CDR2, and CDR3 of the VL sequence of SEQ ID NO: 5.

The present invention further provides an isolated antibody or antigen-binding fragment that specifically binds to human IL-13-Rα2 wherein the antibody comprises: (a) a heavy chain CDR1 comprising SEQ ID NO: 2; (b) a heavy chain CDR2 comprising SEQ ID NO: 3; (c) a heavy chain CDR3 comprising SEQ ID NO: 4; (d) a light chain CDR1 comprising SEQ ID NO: 6; (e) a light chain CDR2 comprising SEQ ID NO: 7; and, (f) a light chain CDR3 comprising SEQ ID NO: 8.

The present invention further provides an isolated antibody or antigen-binding fragment that specifically binds to human IL-13-Rα2 wherein said isolated antibody further comprises the heavy chain variable region amino acid sequence of SEQ ID NO: 1 and the light chain variable region amino acid sequence of SEQ ID NO: 5.

The present invention further provides an isolated antibody or antigen-binding fragment that specifically binds to human IL-13-Rα2 wherein said isolated antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 50 and wherein said isolated antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 51.

The present invention further provides an antibody-drug conjugate comprising a cytotoxic agent conjugated to an antibody or antigen-binding fragment thereof that specifically binds to human IL-13-Rα2,

The present invention further provides an antibody-drug conjugate that specifically binds to human IL-13-Rα2 wherein said conjugate has the formula: Ab-(L-D)*p*, wherein; (a) Ab is the antibody or antigen-binding fragment thereof of the present invention; (b) L-D is a linker-drug moiety, wherein L is a linker, and D is a drug; and (c) *p* is an integer from 1 to about 8.

The present invention further provides an antibody-drug conjugate that specifically binds to human IL-13-Rα2 wherein the linker is selected from the group consisting of maleimidocaproyl (mc) and maleimidocaproyl-Val-Cit-PABA (vc).

The present invention further provides an antibody-drug conjugate that specifically binds to human IL-13-Rα2 wherein the linker-drug moiety has the formula designated vc-0101 or mc-3377 as shown in Example 14.

The present invention further provides an antibody-drug conjugate that specifically binds to human IL-13-Rα2 wherein Ab comprises: (a) a heavy chain variable region comprising a CDR1, CDR2, and CDR3 of the VH sequence of SEQ ID NO: 1; and, (b) a light chain variable region comprising a CDR1, CDR2, and CDR3 of the VL sequence of SEQ ID NO: 5.

The present invention further provides an antibody-drug conjugate that specifically binds to human IL-13-Rα2 wherein Ab comprises: (a) a heavy chain CDR1 comprising SEQ ID NO: 2; (b) a heavy chain CDR2 comprising SEQ ID NO: 3; (c) a heavy chain CDR3 comprising SEQ ID NO: 4; (d) a light chain CDR1 comprising SEQ ID NO: 6; (e) a light chain CDR2 comprising SEQ ID NO: 7; and, (f) a light chain CDR3 comprising SEQ ID NO: 8.

The present invention further provides an antibody-drug conjugate wherein L-D is selected from the group consisting of vc-0101, mc-3377, mc-0131, MalPeg-6121, MalPeg-0131, mc-6121, vc-3906, vc-6780, mc-8261, mc3906, and MalPeg-8261.

The present invention further provides an antibody-drug conjugate that specifically binds to human IL-13-Rα2 wherein said conjugate utilizes site-specific conjugation on engineered cysteine residues and has the formula: Ab-(L-D)*p,* or a pharmaceutically acceptable salt thereof wherein; (a) Ab is the antibody or antigen-binding fragment thereof comprising a heavy chain variable region comprising a CDR1, CDR2, and CDR3 of the VH sequence shown in SEQ ID NO: 1; a light chain variable region comprising a CDR1, CDR2, and CDR3 of the VL sequence shown in SEQ ID NO: 5; and an engineered Fc region comprising at least one pair of amino acid substitutions selected from the group consisting of the amino acid sequence of SEQ ID NO:33 and SEQ ID NO:34; or an engineered Fc region and at least one engineered light chain constant region selected from group consisting of L443C (SEQ ID NO: 28), Q347C (SEQ ID NO: 29), kK183C (SEQ ID NO: 31), L443C/kA111C (SEQ ID NOS: 28 and 30), L443C/kK183C (SEQ ID NOS: 28 and 31), Q347C/kA111C (SEQ ID NOS: 29

and 30), and Q347C/kK183C (SEQ ID NOS: 29 and 31); (b) L-D is a linker-drug moiety, wherein L is a linker, and D is a drug; and (c) *p* is an integer from 1 to about 8.

The present invention further provides the antibody-drug conjugate described above that utilizes site specific conjugation on engineered cysteine residues, wherein the linker-drug moiety has the formula designated vc-0101 or mc-3377 as shown in Example 14, or a pharmaceutically acceptable salt or solvate form thereof, and *p* is an integer of about 4.

The present invention further provides the antibody-drug conjugate of the present invention that utilizes the Multifunctional Antibody Conjugates (MAC) technology, wherein said antibody or antigen binding portion thereof specifically binds to human IL-13Rα2 wherein the antibody has the mutation D185A at position 185 of the LC as shown in SEQ ID NO: 52, and the antibody is covalently conjugated to at least one drug moiety through a linker attached to a side chain of K188 of the LC of SEQ ID NO:49; wherein the drug moiety has the formula designated 0101 or 3377 as shown in Example 13, or a pharmaceutically acceptable salt or solvate form thereof, and *p* is an integer in a range whose lower limit may be selected from the group consisting of about 1.5, about 1.6, about 1.7, about 1.8, about 1.9, and about 2.0, and whose upper limit may be selected from the group consisting of about 2.0, about 2.1, about 2.2, about 2.3, about 2.4, about 2.5. In some aspects, *p* is about 2.

The present invention further provides a pharmaceutical composition comprising an antibody-drug conjugate of the present invention and a pharmaceutically acceptable carrier.

The present invention further provides a method of treating an IL-13-Rα2 expressing cancer in a patient in need thereof, comprising administering to said patient an antibody-drug conjugate of the present invention.

The present invention further provides a method of treating an IL-13-Rα2 expressing cancer wherein said cancer is selected from the group consisting of carcinomas of the bladder, breast, cervix, colon, malignant gliomas, endometrium, kidney, lung, esophagus, ovary, prostate, pancreas, melanoma, stomach, and testes.

More preferably, the present invention provides a method of treating an IL-13-Rα2 expressing cancer wherein said cancer is selected from the group consisting of, lung, colon, stomach, pancreatic, ovarian, malignant gliomas, and melanoma.

The invention further provides an antibody-drug conjugate of the present invention for use in therapy.

The invention further provides use of an antibody-drug conjugate of the present invention for the manufacture of a medicament for therapy.

The invention further provides the use of an antibody-drug conjugate of the present invention, wherein said use is for the treatment of an IL-13-Rα2 expressing cancer.

The invention further provides a nucleic acid that encodes an IL-13-Rα2 antibody, a vector comprising said nucleic acid, and a host cell comprising said vector.

The invention further provides a process for producing an IL-13-Rα2 antibody wherein said process comprises culturing the host cell comprising the above mentioned vector and recovering the antibody from the cell culture.

The invention further provides a process for producing an IL-13-Rα2 antibody-drug conjugate comprising: (a) linking a linker selected from the group consisting of maleimidocaproyl and maleimidocaproyl-Val-Cit-PABA to a drug selected from the group consisting of 0101 and 3377 resulting in a linker-drug moeity; (b) conjugating said linker-drug moeity to the antibody recovered from the cell culture of indicated above; and, (c) purifying the antibody-drug conjugate.

The invention further provides an isolated antibody that competes with an antibody or antigen-binding fragment thereof of the present invention for specific binding to human IL-13-Rα2.

The invention further provides an antibody–drug conjugate comprising an antibody or antigen-binding fragment thereof of the present invention for specific binding to human IL-13-Rα2.

The invention further provides a method for predicting whether a subject with cancer will respond to an antibody-drug conjugate of the present invention comprising:

determining whether a biological sample of said cancer from the subject expresses hIL-13-Rα2.

The invention further provides a process of determining the level of hIL-13-Rα2 in a biological sample comprising the steps of: testing a sample from a subject suspected to have cancer in a immunoassay using an antibody of the present invention; determining the cell surface levels of hIL-13-Rα2 on said sample; and, comparing the cell surface levels of hIL-13-Rα2 with that of a reference subject or standard.

The invention further a method of treating an IL-13-Rα2 expressing cancer said method comprising: determining the level of hIL-13-Rα2 in a biological sample comprising the steps of: testing a sample from a subject suspected to have cancer in a immunoassay using an antibody of the present invention; determining the cell surface levels of hIL-13-Rα2 on said sample; comparing the cell surface levels of hIL-13-Rα2 with that of a normal reference subject or standard; and administering an antibody–drug conjugate of the present invention.

Brief Description of the Drawings

Fig. 1: Binding specificity of chimerc antibodies ch07 and ch08 to hIL-13-Rα2 but not hIL-13Rα1.

Fig. 2A and Fig. 2B: ch07, ch08 and antibodies MAB614 and ab27414 have distinct binding epitopes to IL-13Rα2.

Fig. 2C: Biacore analysis indicating that ch07 and ch08 lack competition in binding.

Fig. 3: ch07 and ch08 are non-neutralizing antibodies.

Fig. 4A – 4G: SEQ ID NOS: 1 – 55.

Detailed Description of the Invention

The present invention provides IL-13-Rα2 antibody-drug conjugates for the treatment of cancer. In order that the present invention is more readily understood, certain terms and general techniques are first defined.

All amino acid abbreviations used in this disclosure are those accepted by the United States Patent and Trademark Office as set forth in 37 C.F.R. § 1.822 (d)(1).

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclature used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art.

The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g., Sambrook J. & Russell D. *Molecular Cloning: A Laboratory Manual,* 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2000); Ausubel et al., *Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*, Wiley, John & Sons, Inc. (2002); Harlow and Lane *Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1998); and Coligan et al., *Short Protocols in Protein Science*, Wiley, John & Sons, Inc. (2003).

An “antibody” or “Ab” is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term “antibody” encompasses not only intact polyclonal or monoclonal antibodies, but also any antigen binding fragment (i.e., "antigen-binding portion") or single chain thereof, fusion proteins comprising an antibody, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site including, for example without limitation, scFv, single domain antibodies (*e.g.*, shark and camelid antibodies), maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (see, e.g., Hollinger and Hudson, 2005,

Nature Biotechnology 23(9): 1126-1136). An antibody includes an antibody of any class, such as IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant region of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant regions that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The term “isolated” refers to a molecule that is substantially free of its natural environment. For instance, an isolated antibody is substantially free of cellular material or other proteins from the cell or tissue source from which it was derived.

The term "antigen-binding fragment" of an antibody, as used herein, refers to one or more fragments of an intact antibody that retain the ability to specifically bind to a given antigen (e.g., target IL-13-Rα2). Antigen binding functions of an antibody can be performed by fragments of an intact antibody. Examples of binding fragments encompassed within the term "antigen binding portion" of an antibody include Fab; Fab’; $F(ab')_2$; an Fd fragment consisting of the VH and CH1 domains; an Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a single domain antibody (dAb) fragment (Ward et al., 1989 Nature 341:544-546), and an isolated complementarity determining region (CDR).

A “variable region” of an antibody refers to the variable region of the antibody light chain (VL) or the variable region of the antibody heavy chain (VH), either alone or in combination. As known in the art, the variable regions of the heavy and light chain each consist of four framework regions (FRs) connected by three complementarity determining regions (CDR1, CDR2, and CDR3) also known as hypervariable regions, contribute to the formation of the antigen binding site of antibodies. If variants of a subject variable region are desired, particularly with substitution in amino acid residues outside of a CDR region (i.e., in the framework region), appropriate amino acid

substitution, preferably, conservative amino acid substitution, can be identified by comparing the subject variable region to the variable regions of other antibodies which contain CDR1 and CDR2 sequences in the same canonincal class as the subject variable region (Chothia and Lesk, J Mol Biol 196(4): 901-917, 1987). When choosing FR to flank subject CDRs, e.g., when humanizing or optimizing an antibody, FRs from antibodies which contain CDR1 and CDR2 sequences in the same canonical class are preferred.

A "CDR" of a variable domain are amino acid residues within the variable region that are identified in accordance with the definitions of the Kabat, Chothia, the acccumulation of both Kabat and Chothia, AbM, contact, and/or conformational definitions or any method of CDR determination well known in the art. Antibody CDRs may be identified as the hypervariable regions originally defined by Kabat et al. See, e.g., Kabat et al., 1992, Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, NIH, Washington D.C. The positions of the CDRs may also be identified as the structural loop structures originally described by Chothia and others. See, e.g., Chothia et al., 1989, Nature 342:877-883. Other approaches to CDR identification include the "AbM definition," which is a compromise between Kabat and Chothia and is derived using Oxford Molecular's AbM antibody modeling software (now Accelrys®), or the "contact definition" of CDRs based on observed antigen contacts, set forth in MacCallum et al., 1996, J. Mol. Biol., 262:732-745. In another approach, referred to herein as the "conformational definition" of CDRs, the positions of the CDRs may be identified as the residues that make enthalpic contributions to antigen binding. See, e.g., Makabe et al., 2008, Journal of Biological Chemistry, 283:1156-1166. Still other CDR boundary definitions may not strictly follow one of the above approaches, but will nonetheless overlap with at least a portion of the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. As used herein, a CDR may refer to CDRs defined by any approach known in the art, including combinations of approaches. The methods used herein may utilize CDRs defined according to any of these approaches. For any given embodiment

containing more than one CDR, the CDRs may be defined in accordance with any of Kabat, Chothia, extended, AbM, contact, and/or conformational definitions.

The terms "IgG Fc region", “Fc region”, “Fc domain” and “Fc”, as interchangeably used herein refer to the portion of an IgG molecule that correlates to a crystallizable fragment obtained by papain digestion of an IgG molecule. The Fc region consists of the C-terminal half of the two heavy chains of an IgG molecule that are linked by disulfide bonds. It has no antigen binding activity but contains the carbohydrate moiety and the binding sites for complement and Fc receptors, including the FcRn receptor (see below). The Fc fragment contains the entire second constant domain CH2 (residues 231-340 of human IgG1, according to the Kabat numbering system) and the third constant domain CH3 (residues 341-447).

By “engineered Fc polypeptide”, “engineered Fc region” and “engineered Fc” as the terms are interchangeably used herein, is meant an Fc polypeptide, or portion thereof, comprising at least one mutation, e.g., an amino acid substitution, introducing a site for conjugation. Preferably, the mutation introduces a cysteine in place of the naturally-occurring amino acid residue at that position, where the mutation creates a reactive site (e.g., a reactive sulfhydryl group) for conjugation of a moiety to the Fc.

The term "monoclonal antibody" or “mAb” refers to an antibody that is derived from a single copy or clone, including e.g., any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. Preferably, a monoclonal antibody of the invention exists in a homogeneous or substantially homogeneous population.

"Humanized" antibody refers to forms of non-human (*e.g.* murine) antibodies that are chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. Preferably, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity.

“Human antibody” or “Fully Human antibody” refers to those antibodies derived from transgenic mice carrying human antibody genes or from human cells.

The term “chimeric antibody” is intended to refer to antibodies in which the variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody in which the variable region sequences are derived from a mouse antibody and the constant region sequences are derived from a human antibody.

A "therapeutic agent" is an agent that exerts a cytotoxic, cytostatic, and/or immunomodulatory effect on cancer cells or activated immune cells. Examples of therapeutic agents include cytotoxic agents, chemotherapeutic agents, cytostatic agents, and immunomodulatory agents.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer.

A "cytotoxic effect" refers to the depletion, elimination and/or the killing of a target cell(s). A "cytotoxic agent" refers to an agent that has a cytotoxic and/or cytostatic effect on a cell.

A "cytostatic effect" refers to the inhibition of cell proliferation. A "cytostatic agent" refers to an agent that has a cytostatic effect on a cell, thereby inhibiting the growth and/or expansion of a specific subset of cells.

“Antibody-drug conjugate” or ”ADC” refers to antibodies or antibody fragments thereof, including antibody derivatives that bind to IL-13-Rα2 and are conjugated to cytotoxic, cytostatic, and/or therapeutic agents.

“Anti- IL-13-Rα2 Antibody-Drug conjugate” refers to an anti- IL-13-Rα2 antibody or antigen binding fragment thereof, as described herein linked to a cytotoxic drug (D) via a linker (L).

“Linker (L)” describes the direct or indirect linkage of the antibody to the drug. Attachment of a linker to a mAb can be accomplished in a variety of ways, such as through surface lysines, reductive-coupling to oxidized carbohydrates, and through cysteine residues liberated by reducing interchain disulfide linkages. A variety of ADC

linkage systems are known in the art, including hydrazone-, disulfide- and peptide-based linkages.

"Drug (D)" is any substance having biological or detectable activity, for example, therapeutic agents, detectable labels, binding agents, etc., and prodrugs, which are metabolized to an active agent *in vivo*. The terms drug, drug moiety, payload, and compound are used interchangeably.

"L-D" is a linker-drug moiety resulting from a cytotoxic drug (D) linked to a linker (L).

The term "epitope" refers to that portion of a molecule capable of being recognized by and bound by an antibody at one or more of the antibody's antigen-binding regions. Epitopes often consist of a chemically active surface grouping of molecules such as amino acids or sugar side chains and have specific three-dimensional structural characteristics as well as specific charge characteristics. The term "antigenic epitope" as used herein, is defined as a portion of a polypeptide to which an antibody can specifically bind as determined by any method well known in the art, for example, by conventional immunoassays. A "nonlinear epitope" or "conformational epitope" comprises noncontiguous polypeptides (or amino acids) within the antigenic protein to which an antibody specific to the epitope binds. Once a desired epitope on an antigen is determined, it is possible to generate antibodies to that epitope, e.g., using the techniques described in the present specification. During the discovery process, the generation and characterization of antibodies may elucidate information about desirable epitopes. From this information, it is then possible to competitively screen antibodies for binding to the same epitope. An approach to achieve this is to conduct competition and cross-competition studies to find antibodies that compete or cross-compete with one another *e.g.*, the antibodies compete for binding to the antigen.

The term "binding affinity (K_D)" as used herein, is intended to refer to the dissociation rate of a particular antigen-antibody interaction. The K_D is the ratio of the rate of dissociation (K_d), also called the "off-rate (k_{off})", to the association rate (K_a), or "on- rate (k_{on})". Thus, K_D equals k_{off} / k_{on} and is expressed as a molar concentration (M). It follows that the smaller the K_D, the stronger the affinity of binding. Therefore, a K_D

of 1 μM indicates weak binding affinity compared to a K_D of 1 nM. K_D values for antibodies can be determined using methods well established in the art. One method for determining the K_D of an antibody is by using surface plasmon resonance, typically using a biosensor system such as a Biacore® system.

The term “specifically binds” as used herein in reference to the binding between an antibody and an IL-13-Rα2 antigen and the antibody binds the IL-13-Rα2 antigen with a K_D less than about 30 nM as determined by surface plasmon resonance (SPR) at 25°C.

“Pharmaceutically acceptable salt” as used herein refers to pharmaceutically acceptable organic or inorganic salts of a molecule or macromolecule.

The term "potency" is a measurement of biological activity and may be designated as IC_{50}, or inhibitory concentration of an antibody or antibody drug conjugate to the antigen IL-13-Rα2, needed to inhibit 50% of growth of an IL-13-Rα2 positive cell line as described in Example 15.

“EC50” is a measurement of binding capacity and is defined as the half maximal effective concentration of an antibody or antibody-drug conjugate that is needed to produce a response halfway between the baseline and maximum.

The phrase “effective amount” or “therapeutically effective amount” as used herein refers to an amount necessary (at dosages and for periods of time and for the means of administration) to achieve the desired therapeutic result. An effective amount is at least the minimal amount, but less than a toxic amount, of an active agent which is necessary to impart therapeutic benefit to a subject.

The terms “inhibit” or “neutralize” as used herein with respect to bioactivity of an antibody of the invention mean the ability of the antibody to substantially antagonize, prohibit, prevent, restrain, slow, disrupt, eliminate, stop, reduce or reverse e.g. progression or severity of that which is being inhibited including, but not limited to , a biological activity.

The term “compete”, as used herein with regard to an antibody, means that a first antibody, or an antigen-binding portion thereof, binds to an epitope in a manner sufficiently similar to the binding of a second antibody, or an antigen-binding portion

thereof, such that the result of binding of the first antibody with its cognate epitope is detectably decreased in the presence of the second antibody compared to the binding of the first antibody in the absence of the second antibody. The alternative, where the binding of the second antibody to its epitope is also detectably decreased in the presence of the first antibody, can, but need not be the case. That is, a first antibody can inhibit the binding of a second antibody to its epitope without that second antibody inhibiting the binding of the first antibody to its respective epitope. However, where each antibody detectably inhibits the binding of the other antibody with its cognate epitope or ligand, whether to the same, greater, or lesser extent, the antibodies are said to “cross-compete” with each other for binding of their respective epitope(s). Both competing and cross-competing antibodies are encompassed by the present invention. Regardless of the mechanism by which such competition or cross-competition occurs (e.g., steric hindrance, conformational change, or binding to a common epitope, or portion thereof), the skilled artisan would appreciate, based upon the teachings provided herein, that such competing and/or cross-competing antibodies are encompassed and can be useful for the methods disclosed herein.

The terms “polynucleotide” or "nucleic acid molecule", as used herein, are intended to include DNA molecules and RNA molecules. A nucleic acid molecule may be single-stranded or double-stranded, but preferably is double-stranded DNA.

The polynucleotides that encode the antibodies of the present invention may include the following: only the coding sequence for the variant, the coding sequence for the variant and additional coding sequences such as a functional polypeptide, or a signal or secretory sequence or a pro-protein sequence; the coding sequence for the antibody and non-coding sequence, such as introns or non-coding sequence 5’ and/or 3’ of the coding sequence for the antibody. The term ‘polynucleotide encoding an antibody” encompasses a polynucleotide which includes additional coding sequence for the variant but also a polynucleotide which includes additional coding and/or non-coding sequence. It is known in the art that a polynucleotide sequence that is optimized for a specific host cell/expression system can readily be obtained from the amino acid sequence of the desired protein (see GENEART AG, Regensburg, Germany).

A “host cell” includes an individual cell or cell culture that can be or has been a recipient for vector(s) for incorporation of polynucleotide inserts. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected in vivo with a polynucleotide(s) of this invention.

The term "vector" means a construct, which is capable of delivering, and, preferably, expressing, one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors, plasmid, cosmid or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, DNA or RNA expression vectors encapsulated in liposomes, and certain eukaryotic cells, such as producer cells.

The term "expression control sequence" means a nucleic acid sequence that directs transcription of a nucleic acid. An expression control sequence can be a promoter, such as a constitutive or an inducible promoter, or an enhancer. The expression control sequence is operably linked to the nucleic acid sequence to be transcribed.

The polynucleotides encoding the antibodies of the present invention will typically include an expression control polynucleotide sequence operably linked to the antibody coding sequences, including naturally-associated or heterologous promoter regions known in the art. Preferably, the expression control sequences will be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells, but control sequences for prokaryotic hosts may also be used. Once the vector has been incorporated into the appropriate host cell line, the host cell is propagated under conditions suitable for expressing the nucleotide sequences, and, as desired, for the collection and purification of the antibodies. Preferred eukaryotic cell lines include the CHO cell lines, various COS cell lines, HeLa cells, myeloma cell lines, transformed B-cells, or human embryonic kidney cell lines. The most preferred host cell is a CHO cell line.

Antibodies

Antibodies of the invention can be produced using techniques well known in the art, *e.g.*, recombinant technologies, phage display technologies, synthetic technologies or combinations of such technologies or other technologies readily known in the art (see, for example, Jayasena, S.D., Clin. Chem., 45: 1628-50 (1999) and Fellouse, F.A., et al, J. Mol. Biol., 373(4):924-40 (2007)).

Tables 1 and 2 below depict preferred CDRs for the antibodies of the present invention.

Table 1

Antibody	LCDR1	LCDR2	LCDR3
hu07	TASLSVSSTYLH SEQ ID NO: 14	STSNLAS SEQ ID NO: 15	HQYHRSPLT SEQ ID NO: 16
hu08	KASQDVGTAVA SEQ ID NO: 6	SASYRST SEQ ID NO: 7	QHHYSAPWT SEQ ID NO: 8

Table 2

Antibody	HCDR1	HCDR2	HCDR3
hu07	TKYGVH SEQ ID NO: 10	VKWAGGSTDYNSALMS SEQ ID NO: 11	DHRDAMDY SEQ ID NO: 12
hu08	SRNGMS SEQ ID NO: 2	TVSSGGSYIYYADSVKG SEQ ID NO: 3	QGTTALATRFFDV SEQ ID NO: 4

An embodiment of the present invention includes an antibody or antigen binding fragment thereof, that comprises:

a) a light chain variable region comprising:

i) a LCDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6 and 14;

ii) a LCDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 7 and 15; and

iii) a LCDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8 and 16; and

b) a heavy chain variable region comprising:

i) a HCDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 10;

ii) a HCDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 3 and 11; and

iii) a HCDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4 and 12.

A preferred antibody or antigen binding portion thereof, of the invention comprises:

a) a LCVR comprising: a LCDR1 of SEQ ID NO: 6, a LCDR2 of SEQ ID NO: 7, and a LCDR3 of SEQ ID NO: 8; and

b) a HCVR comprising: a HCDR1 of SEQ ID NO: 2, a HCDR2 of SEQ ID NO: 3, and a HCDR3 of SEQ ID NO: 4.

Another preferred antibody or antigen binding portion thereof, of the invention comprises:

a) a LCVR comprising: a LCDR1 of SEQ ID NO: 14, a LCDR2 of SEQ ID NO: 15, and a LCDR3 of SEQ ID NO: 16; and

b) a HCVR comprising: a HCDR1 of SEQ ID NO: 10, a HCDR2 of SEQ ID NO: 11, and a HCDR3 of SEQ ID NO: 12.

Preferred monoclonal antibodies of the invention are referred to herein as hu08 (a humanized anti- IL-13-Rα2 IgG1 antibody); and, hu07 (a humanized anti- IL-13-Rα2 IgG1 antibody). The SEQ ID NOs of the amino acid sequences encoding mAbs hu08 and hu07 are provided in Table 3 below:

Table 3

mAb	LC	HC	LC VR	LCDR 1	LCDR 2	LCDR 3	HCV R	HCDR 1	HCDR 2	HCDR 3
hu08	51	50	5	6	7	8	1	2	3	4
hu07	53	52	41	14	15	16	48	10	11	12

An embodiment of the invention is an antibody or antigen binding fragment thereof that specifically binds to the same IL-13Rα2 epitope as an antibody comprising a first amino acid sequence that is at least 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 1 and a second amino acid sequence that is at least 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 5.

Another embodiment of the invention is an antibody or antigen binding fragment thereof that specifically binds to the same IL-13Rα2 epitope as an antibody comprising a first amino acid sequence that is at least 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 48 and a second amino acid sequence that is at least 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 41.

In some embodiments, the antibody or antigen binding fragment thereof specifically binds to IL-13Rα2, and the antibody or fragment thereof competitively inhibits the binding of an antibody comprising a first amino acid sequence that is at least 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 1 and a second amino acid sequence that is at least 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 5.

In some embodiments, the antibody or antigen binding fragment thereof specifically binds to IL-13Rα2, and the antibody or fragment thereof competitively inhibits the binding of an antibody comprising a first amino acid sequence that is at least 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 48 and a second amino acid sequence that is at least 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 41.

Representative materials of the present invention were deposited in the American Type Culture Collection (ATCC) on November 6, 2012. A vector having ATCC Accession No. PTA-13304 is a polynucleotide encoding a human anti-IL-13 antibody light chain variable region designated as hu08-VLv1.0, and vector having ATCC Accession No. PTA-13305 is a polynucleotide encoding a human anti-IL-13 antibody heavy chain variable region, designated hu08-VHv1.0. The deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposit will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Pfizer, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 U.S.C. Section 122 and the Commissioner's rules pursuant thereto (including 37 C.F.R. Section 1.14 with particular reference to 886 OG 638).

Conjugation of Drug moieties to an Antibody

The drug moiety has, or is modified to include, a group reactive with a conjugation point on the antibody. For example, a drug moiety can be attached by alkylation (*e.g.*, at the epsilon-amino group lysines or the N-terminus of antibodies), reductive amination of oxidized carbohydrate, transesterification between hydroxyl and carboxyl groups, amidation at amino groups or carboxyl groups, and conjugation to thiols. In some embodiments, the number of drug moieties, *p*, conjugated per antibody molecule ranges from an average of 1 to 8; 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, or 1 to 2. In some embodiments, *p* ranges from an average of 2 to 8, 2 to 7, 2 to 6, 2 to 5, 2 to 4 or 2 to 3. In other embodiments, *p* is an average of 1, 2, 3, 4, 5, 6, 7 or 8. In some embodiments, *p* ranges from an average of about 1 to about 8; about 1 to about 7,

about 1 to about 6, about 1 to about 5, about 1 to about 4, about 1 to about 3, or about 1 to about 2. In some embodiments, *p* ranges from about 2 to about 8, about 2 to about 7, about 2 to about 6, about 2 to about 5, about 2 to about 4 or about 2 to about 3. For examples of chemistries that can be used for conjugation, see, *e.g.*, Current Protocols in Protein Science (John Wiley & Sons, Inc.), Chapter 15 (Chemical Modifications of Proteins).

Linkers

The drug moiety can be linked to an antibody by a linker. Suitable linkers include, for example, cleavable and non-cleavable linkers. A cleavable linker is typically susceptible to cleavage under intracellular conditions. Suitable cleavable linkers include, for example, a peptide linker cleavable by an intracellular protease, such as lysosomal protease or an endosomal protease. In exemplary embodiments, the linker can be a dipeptide linker, such as a valine-citrulline (val-cit), a phenylalanine-lysine (phe-lys) linker, or maleimidocapronic –valine-citruline-p-aminobenzyloxycarbonyl (vc) linker. Another linker is Sulfosuccinimidyl-4-[N-maleimidomethyl]cyclohexane-1-carboxylate (smcc). Sulfo-smcc conjugation occurs via a maleimide group which reacts with sulfhydryls (thiols, -SH), while its Sulfo-NHS ester is reactive toward primary amines (as found in Lysine and the protein or peptide N-terminus). Yet another linker is maleimidocaproyl (mc). Other suitable linkers include linkers hydrolyzable at a specific pH or a pH range, such as a hydrazone linker. Additional suitable cleavable linkers include disulfide linkers. The linker may be covalently bound to the antibody to such an extent that the antibody must be degraded intracellularly in order for the drug to be released *e.g.* the mc linker and the like.

The preferred linkers of the present invention are maleimidocapronic –valine-citruline-p-aminobenzyloxycarbonyl (vc) and maleimidocaproyl (mc).

Engineered Fc Polypeptide

It has been previously reported that certain residues presumably present on the surface of the CH2 or CH3 domain of the heavy chain of antibodies, or on the constant domain of the light chain, or otherwise accessible, are suitable for the substitution of the naturally-occurring wild type amino acid with, for example, cysteine, and are therefore

useful to engineer a site capable of conjugation to various agents (see US Provisional Patent Application USSN 61/580169) herein incorporated by reference.

Amino acid modifications can be made by any method known in the art and many such methods are well known and routine for the skilled artisan. For example, but not by way of limitation, amino acid substitutions, deletions and insertions may be accomplished using any well-known PCR-based technique. Amino acid substitutions may be made by site-directed mutagenesis (see, for example, Zoller and Smith, 1982, Nucl. Acids Res. 10:6487-6500; an d Kunkel, 1985, Proc. Natl. Acad. Sci USA 82:488).

In some embodiments, the engineered Fc polypeptide of the disclosure may be used to prepare an antibody, or antigen binding fragment thereof, such that the antibody or fragment thereof thereby comprises the engineered Fc region which can be used to conjugate, at the engineered residue (i.e., the amino acid substituted compared to wild type unmodified Fc), a wide variety of moieties.

In some embodiments, the engineered kappa light chain constant polypeptide of the disclosure may be used to prepare an antibody, or antigen binding fragment thereof, such that the antibody or fragment thereof thereby comprises an engineered CL region comprising an amino acid mutation, or portion thereof, which can be used to conjugate, at the engineered amino acid residue, a wide variety of moieties.

The IL-13-Rα2 antibodies of the present invention may encompass an engineered Fc polypeptide where 1, 2, or more amino acids chosen from positions: 347, 392, 398, 422 and 443 of the antibody heavy chain wherein the numbering system of the constant region is that of the EU index as set forth in Kabat et al. (1991, NIH Publication 91- 3242, National Technical Information Service, Springfield, VA, hereinafter "Kabat") of a parent, native, or wild type antibody, substituted with another amino acid (including natural and non-natural/synthetic amino acids).

It should be noted that a single substitution in an Fc polypeptide, for example of a cysteine residue, normally results in the display of two corresponding residues in the resultant IgG antibody due to the homodimeric nature of IgG antibody molecules. Thus, the resultant engineered IgG antibodies of the invention may display at least 1, 2, 3, 4, or more reactive groups for the purpose of conjugation to a drug or compound. In an

embodiment, one or more of the substitutions is with a cysteine residue, and the resulting engineered antibodies may display at least 1, 2, 3, 4, or more thiol groups for the purpose of conjugation to a drug or compound.

In other embodiments, the engineered Fc polypeptide of the disclosure comprises one or more substitutions selected from the positions 347, 392, 398, 422 and 443, of the heavy chain of an antibody, and wherein the numbering system of the constant region is that of the EU index as set forth in Kabat et al. (supra).

In some embodiments, the engineered Fc polypeptide of the disclosure comprises at least one pair of amino acid substitutions selected from the group consisting of: (a) the amino acid sequence of SEQ ID NO:33; and, (b) the amino acid sequence of SEQ ID NO:34.

In some embodiments, the engineered Fc polypeptide of the disclosure comprises one substitution selected from the group consisting of (a) the amino acid sequence of SEQ ID NO:28; and (b) the amino acid sequence of SEQ ID NO:29.

Engineered Cκ polypeptide

The IL-13-Rα2 antibodies of the present invention may encompass an engineered antibody light chain constant region (LC), or a portion thereof, where 1, 2, or 3 amino acids chosen from positions 111, 183, or 188, of the antibody light chain, wherein the numbering system of the light chain constant region is that of the Kabat numbering system as set forth in Kabat et al. (1991, NIH Publication 91- 3242, National Technical Information Service, Springfield, VA, hereinafter "Kabat"), of a parent, native, or wild type antibody, substituted with another amino acid (including natural and non-natural/synthetic amino acids).

In some embodiments, the engineered LC polypeptide of the disclosure comprises one or more substitutions selected from the group consisting of (a) the amino acid sequence of SEQ ID NO:30; (b) the amino acid sequence of SEQ ID NO:31; and (c) the amino acid sequence of SEQ ID NO:32.

In other embodiments, due to the dimeric nature of many antibodies (e.g., IgGs comprise two light chains and two heavy chains each heavy chain comprising an Fc polypeptide), an antibody of the invention may comprise at least one engineered Fc

polypeptide and may further comprise at least one engineered light chain constant polypeptide thereby providing at least two site-specific conjugation sites – one in the Fc polypeptide and another in the CL polypeptide. Preferred antibodies of the invention that comprise at least one engineered Fc polypeptide and at least one engineered light chain constant region polypeptide selected from group consisting of L443C/kA111C (SEQ ID NOS: 28 and 30), L443C/kK183C (SEQ ID NOS: 28 and 31), Q347C/kA111C (SEQ ID NOS: 29 and 30), and Q347C/kK183C (SEQ ID NOS: 29 and 31).

MAC Conjugation Technology

The term multifunctional antibody conjugate, or MAC, refers to an antibody as defined herein, or antigen binding portion thereof, covalently conjugated through the constant kappa region to at least one drug moiety that exerts a biological effect to a target. Preferably, the antibody, or antigen binding portion thereof comprises K90 and H91 of SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, or SEQ ID NO:55, and the drug moiety is conjugated at K90. MAC technology has been described previously in WO2012/007896 and in USSN 61/584,675, which are incorporated herein by reference.

The drug moiety exerts a biological effect on the target and may be a peptide, small molecule, protein, nucleic acid molecule, toxin, aptamer, or antigen binding antibody or fragment thereof. The drug moiety may be a drug having cytotoxic activity against target cells. In some aspects, the cytotoxin is in the class of compounds known as auristatin. Representative auristatins are compounds 0101 and 3377 described herein in Example 13.

Reaction of the drug moiety with the constant light domain of an antibody is particularly desirable to minimize, or prevent, any interference with binding of the Fc portion of the antibody to Fc receptors (such as FcγR and FcRn) or binding of the antibody to its respective target. Conversely, conjugation of the respective drug moiety to the Fc portion of an antibody may decrease the antibody half-life *in vivo* and/or its capacity to interact with the immune system (effector function). Conjugation of the drug moiety in the variable heavy chain (VH) or variable light chain (VL) region of the antibody carry a risk of diminishing the binding of the antibody to its cognate.

One of the advantages of the MAC technology is that depending on the reagents and reaction conditions (especially the leaving group ester and molar ratio of linker antibody), compositions and samples of the invention can be generated with a defined number of drug moieties relative to a defined number of antibodies. This can be especially useful when balancing the relative reactivity's and therapeutic windows of the drug moiety and antibody. Moreover, in some situations, increasing the number of drug moieties per antibody beyond a certain threshold may not result in increased target binding or therapeutic effect. It is useful therefore, to be able to control the number of drug moieties conjugated per antibody, and in doing so, direct the location of conjugation so as to minimize Fc or combining site interference. In some situations, therefore, aspects of the invention that allow for reduced conjugation, preferentially decorating only a single lysine residue, such as K90 of the hu08 LC constant region, SEQ ID NO: 52, SEQ ID NO:53, SEQ ID NO:54, or SEQ ID NO:55, can be advantageous. Furthermore, whereas conjugation to K90 is reliable and robust, conjugation to other antibody surface lysines, each of slightly different reactivity and pI can result in an heterogeneous sample of conjugated antibodies that can release conjugated molecules at inopportune or irregular times, such as during circulation and prior to delivery of the drug moiety to the target by antibody recognition.

A further aspect of the present invention is the discovery that certain mutations of D77 of the wild type constant kappa chain (SEQ ID NO: 55) improves the accessibility and/or reactivity of the K90 site for drug conjugation. In addition, the present invention provides for known polymorphisms of the kappa chain V/A at position 45 and A/L at position 83 (giving the 3 identified human constant kappa polymorphisms Km(1):V45/L83, Km(1,2): A45/L83, and Km(3) A45/V83). Accordingly, the present invention provides for MACs comprising SEQ ID NO:53. In some aspects, the present invention provides for a MAC of Km(3) polymorphism, wherein the kappa constant domain is selected from the group consisting of SEQ ID NO:52, SEQ ID NO:54 and SEQ ID NO:55.

The present invention further provides an antibody that specifically binds to human IL-13Rα2 wherein said antibody has a LC constant region as shown in SEQ ID

NO: 52, said LC constant region having a lysine residue at position 80 (K80) and an alanine residue substituted for an aspartic acid residue at position 77 (D77A).

The present invention further provides an antibody that specifically binds to human IL-13Rα2 wherein said antibody has a LC constant region as shown in SEQ ID NO: 53, wherein position 45 is V or A, position 83 is A or L, and position 77 is selected from the group consisting of A, G, I, V, L, R, S, T, Q, P, N, M, H and W. In some aspects, where position 45 is V, position 83 is L. In some aspects of SEQ ID NO:53, position 77 is selected from the group consisting of A, G, I, V, L, R, S, T, Q, P, N, M, H and W. The variability of residues at positions 45 and 83 in SEQ ID NO:53 may be selected so as to only provide for any one, two or all three of the Km(1), Km(1,2), and Km(3) polymorphisms.

The present invention further provides an antibody that specifically binds to human IL-13Rα2 wherein said antibody has a LC constant region as shown in SEQ ID NO: 54, wherein position 77 is selected form the group consisting of A, G, I, V, L, R, S, T, Q, P, N, M, H and W.

The present invention further provides an antibody that specifically binds to human IL-13Rα2 wherein said antibody has a LC constant region as shown in SEQ ID NO: 55.

Therapy for Cancer

Cancers, including, but not limited to, a tumor, metastasis, or other disease or disorder characterized by uncontrolled cell growth, can be treated or prevented by administration of an antibody-drug conjugate of the present invention.

Exemplary anti-IL-13-Rα2 ADCs are useful for treating cancer in which IL-13-Rα2 is expressed or overexpressed, relative to normal (e.g., non-cancerous tissue). Treatment or prevention of an IL-13-RA2 -expressing cancer, according to the methods described herein, can be achieved by administering to a subject in need of such treatment an effective amount of an anti-IL-13-Rα2 ADC. In some embodiments, an anti-IL-13-Rα2 full length antibody or antigen-binding fragment thereof or derivative thereof that is conjugated to a cytotoxic agent will be administered. In some exemplary embodiments, an ADC of the present invention will (i) bind to IL-13-Rα2 expressing

cancer cells, and (ii) exert a cytotoxic or cytostatic effect to, for example, inhibit the proliferation of the IL-13-Rα2 expressing cancer cells, or kill IL-13-Rα2 expressing cancer cells.

In other embodiments, the anti-IL-13-Rα2 ADCs are co-administered with another therapeutic agent, or administered sequentially with another therapeutic agent. In some embodiments, the anti-IL-13-Rα2 ADCs are co-administered with chemotherapeutics, including standard of care chemotherapeutics, or administered sequentially.

In some embodiments, the other therapeutic agent will be an agent that is standard of care for the specific disease to be treated or is part of a salvage regimen for the specific disease to be treated. Anti-cancer agents and chemotherapeutic regimens include, for example, anti-cancer antibodies, including, for example, anti-CD52 antibodies (e.g., Alemtuzumab), anti-CD20 antibodies (e.g., Rituximab), and anti-CD40 antibodies (e.g., SGN40); chemotherapeutic regimens including, for example, CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone); CVP (cyclophosphamide, vincristine, and prednisone); RCVP (Rituximab+CVP); RCHOP (Rituximab+CHOP); RICE (RituximAb+ifosamide, carboplatin, etoposide); RDHAP, (Rituximab+dexamethasone, cytarabine, cisplatin); RESHAP (Rituximab+etoposide, methylprednisolone, cytarabine, cisplatin); gemcitabine; combination treatment with vincristine, prednisone, and anthracycline, with or without asparaginase; combination treatment with daunorubicin, vincristine, prednisone, and asparaginase; combination treatment with teniposide and Ara-C (cytarabine); combination treatment with methotrexate and leucovorin; combination treatment with bleomycin, doxorubicin, etoposide, mechlorethamine, prednisone, vinblastine, and vincristine; small molecule inhibitors; and proteosome inhibitors including, for example, bortezomib.

In some embodiments, methods for treating cancer including administering to a patient in need thereof an effective amount of an anti-IL-13-Rα2 ADC in combination with radiation treatment, and optionally another therapeutic agent. In some embodiments, the anti-IL-13-Rα2 ADC is administered concurrently or sequentially with an anti-cancer agent (e.g., a chemotherapeutic agent) and/or with radiation therapy. In

some embodiments, the chemotherapeutic agent or radiation therapy is administered at least an hour, five hours, 12 hours, a day, a week, a month, several months (e.g., up to three months), prior or subsequent to administration of a compound of the present invention.

The ADCs of the present invention can be in the form of a pharmaceutical composition for administration that are formulated to be appropriate for the selected mode of administration, and pharmaceutically acceptable diluent or excipients, such as buffers, surfactants, preservatives, solubilizing agents, isotonicity agents, stabilizing agents, carriers, and the like. Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton Pa., 18th ed., 1995, provides a compendium of formulation techniques as are generally known to practitioners.

These pharmaceutical compositions may be administered by any means known in the art that achieve the generally intended purpose to treat cancer. The preferred route of administration is parenteral, defined herein as referring to modes of administration that include but not limited to intravenous, intramuscular, intraperitoneal, subcutaneous, and intraarticular injection and infusion. The dosage administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired.

Compositions within the scope of the invention include all compositions wherein an ADC is present in an amount that is effective to achieve the desired medical effect for treating cancer. While individual needs may vary from one patient to another, the determination of the optimal ranges of effective amounts of all of the components is within the ability of the clinician of ordinary skill.

Diagnostic

The antibodies or antibody fragments of the invention can also be used to detect hIL-13-Rα2 in a biological sample in vitro or in vivo. In one embodiment, the anti- hIL-13-Rα2 antibodies of the invention are used to determine the level of hIL-13-Rα2 in a tissue or in cells derived from the tissue. In a preferred embodiment, the tissue is a diseased tissue. In a preferred embodiment of the method, the tissue is a tumor or a biopsy thereof. In a preferred embodiment of the method, a tissue or a biopsy thereof is

first excised from a patient, and the levels of hIL-13-Rα2 in the tissue or biopsy can then be determined in an immunoassay with the antibodies or antibody fragments of the invention. The tissue or biopsy thereof can be frozen or fixed. The same method can be used to determine other properties of the hIL-13-Rα2 protein, such as its level of cell surface levels, or cellular localization.

The above-described method can be used to diagnose a cancer in a subject known to or suspected to have a cancer, wherein the level of hIL-13-Rα2 measured in said patient is compared with that of a normal reference subject or standard. Said method can then be used to determine whether a tumor expresses hIL-13-Rα2, which may suggest that the tumor will respond well to treatment with the antibody-drug conjugates of the present invention. Preferably, the tumor is a cancer of the lung, colon, stomach, pancreatic, ovarian, malignant gliomas, and melanoma, or other carcinomas in which hIL-13-Rα2 is expressed, and other cancers yet to be determined in which hIL-13-Rα2 is expressed predominantly.

An embodiment of the invention is a method of treating an IL-13-Rα2 expressing cancer said method comprising: determining the level of hIL-13-Rα2 in a biological sample comprising the steps of: obtaining a sample from a subject suspected to have cancer; testing said sample in a immunoassay using an antibody of the present invention; determining the cell surface levels of hIL-13-Rα2 on said sample; comparing the cell surface levels of hIL-13-Rα2 with that of a normal reference subject or standard; and administering an antibody–drug conjugate of the present invention to said subject.

The present invention further provides for monoclonal antibodies, humanized antibodies and epitope-binding fragments thereof that are further labeled for use in research or diagnostic applications. In preferred embodiments, the label is a radiolabel, a fluorophore, a chromophore, an imaging agent or a metal ion.

A method for diagnosis is also provided in which said labeled antibodies or epitope-binding fragments thereof are administered to a subject suspected of having a cancer, and the distribution of the label within the body of the subject is measured or monitored.

Kit

The present invention also includes kits, e. g. comprising a described cytotoxic conjugate and instructions for the use of the cytotoxic conjugate for killing of particular cell types. The instructions may include directions for using the cytotoxic conjugates in vitro, in vivo or ex vivo. Typically, the kit will have a compartment containing the cytotoxic conjugate. The cytotoxic conjugate may be in a lyophilized form, liquid form, or other form amendable to being included in a kit. The kit may also contain additional elements needed to practice the method described on the instructions in the kit, such a sterilized solution for reconstituting a lyophilized powder, additional agents for combining with the cytotoxic conjugate prior to administering to a patient, and tools that aid in administering the conjugate to a patient.

All publications and patent documents cited above or in the following examples are hereby incorporated by reference in their entirety for all purposes to the same extent as if each were so individually denoted.

The invention will be further described with reference to the following examples; however, it is to be understood that the invention is not limited to such examples.

The following examples of specific aspects for carrying out the present invention are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

Example 1

Generation and evaluation of murine anti-IL-13Rα2 antibodies mu07 and mu08

Anti-hIL-13Rα2 antibodies were prepared in mice using human IL-13Rα2 antigen and standard methods for immunization. (Zhang, C., *Antibody Methods and Protocols*, Methods in Molecular Biology, vol. 901, DOI 10.1007/978-1-61779-931-0_7, © Springer Science+Business Media, LLC 2012). Two murine antibodies, mu07 and mu08, were identified that bound to A375 cells, a melanoma cell line which endogenously expresses high levels of IL-13Rα2 on the cell surface.

An important characteristic of an antibody in an ADC is rapid internalization after binding to its receptor. The antibodies mu07 and mu08 were evaluated and were found to be internalized 38% and 31% respectively, after a1 hour incubation with A375 cells at 37°C.

Example 2

Variable regions of murine anti-IL-13Rα2 antibodies mu07 and mu08

The mu07and mu08 anti-IL-13Rα2 antibody heavy chain and light chain variable regions were cloned using the SMARTer® cDNA synthesis system (Clontech Laboratories Inc. of Mountain View, Calif.) followed by PCR amplification. The cDNA was synthesized by standard techniques and amplified by PCR using a primer which anneals to the SMARTer® IIA oligo sequence and mouse constant region specific primer (mouse Kappa for the light chain and mouse IgG1 for the heavy chain) with PCR SuperMix High Fidelity (Invitrogen, Carlsbad, CA.). Heavy chain and light chain variable region PCR products were subcloned into the pCR4-TOPO vector (Invitrogen, Carlsbad, CA) and the nucleic acid sequence was determined.

The amino acid sequences of the mu07 and mu08 heavy chain variable regions are set forth as amino acid residues of SEQ ID NO:25 and amino acid residues of SEQ ID NO:23, respectively. The amino acid sequences of the mu07and mu08 light chain variable regions are set forth in SEQ ID NO:26 and SEQ ID NO:24, respectively.

Example 3

Binding Specificity and Binding Kinetics of Chimeric Antibodies ch07 and ch08

Chimeric antibodies 07 and 08 (ch07 and ch08) were constructed having murine heavy chain and light chain variable region sequences with human IgG1 heavy chain constant regions and human kappa light chain constant regions using methods known in the art. To assess the binding activity and specificity of ch07 and ch08, a standard direct ELISA protocol was performed utilizing recombinant hIL-13-Rα2 and hIL-13Ra1, receptors for IL-13 cytokine. The binding was detected by horseradish peroxidase (HRP) conjugated goat anti-human IgGKappa. The results in Figure 1 demonstrate that

both chimeric antibodies can bind specifically to hIL-13Rα2 but not hIL-13Rα1. The ED50 is 0.15nM and 0.076nM for ch07 and ch08, respectively.

To assess the binding kinetics of the ch07 and ch08 antibodies, SPR (Surface Plasmon Resonance) experiments were conducted on a Biacore® T100 or T200 instrument using a Biacore® human Fab Capture Kit (GE Healthcare). All data was analyzed using the Biacore® T100 evaluation software version 2.0 with a 1:1 Langmuir binding model.

K_a, K_d and KD are shown on Table 5. At pH7.4, binding affinity to hIL-13-Rα2 for both ch07 and ch08 are in the pM range, 648pM and 964pM, respectively. ch07 dissociation from hIL-13-Rα2 is about 2 fold slower than ch08. At pH6.0, ch07 and ch08 binding affinity to hIL-13-Rα2 are in the low nM range. Dissociation rates of ch08 and ch07 are very similar. Higher KDs at pH6.0 than pH7.4 are due to a slower association rate.

Table 5: Kinetics analysis of chimeric antibody ch07 and ch08

	Ka (1/Ms)	Kd (1/s)	KD(M)
ch07-pH 7.4	3.61E+05	2.34E-04	6.48E-10
ch07-pH 6.0	6.43E+04	3.37E-04	5.24E-9
ch08-pH 7.4	4.32E+05	4.16E-04	9.64E-10
ch08-pH 6.0	7.12E+04	3.81E-04	5.36E-9

Example 4

Binding Epitopes of Antibodies ch07 and ch08

A competition ELISA was performed to examine whether ch07 and ch08 have distinct binding epitopes. Prior to the competition ELISA experiment, ch07 and ch08 were biotinylated. The EC50 of the biotinylated ch07 (biotin-ch07) and ch08 (biotin-ch08) were determined by direct standard ELISA. For the competition ELISA, recombinant hIL-13-Rα2 was coated onto 96-well plates at 50ul of 2μg/mL in PBS overnight at 4℃. The plates were then blocked and washed following a standard ELISA protocol. 3-fold serially diluted ch07 and ch08 (2x final concentration) were

mixed with a constant amount of biotin-ch07 (FIG 2A) or biotin-ch08 (FIG 2B), respectively and were added to the plate and incubated for 1 hour at room temperature. The amount of biotinylated chimeric antibody bound was detected by HRP conjugated streptavidin at 1:5000 for 1 hour. The results are shown in FIG 2. Unlabelled chimeric ch07 competes in binding to hIL-13-Rα2 with biotin-ch07 while unlabelled ch08 shows no sign of competition (FIG 2A). Similar results are obtained when the same set of antibodies were used to compete with biotin-ch08 (FIG 2B). This clearly demonstrates that antibodies ch07 and ch08 have distinct binding epitopes to hIL-13Rα2.

The competition ELISA was also performed with two commercially available antibodies, monoclonal mouse IgG1, MAB614 (R&D Systems) and monoclonal mouse IgG1, ab27414 (Abcam). FIG 2A and Fig 2B show that both commercial antibodies do not compete with either biotin-ch07 or biotin-ch08 for binding to IL-13Rα2, indicating that antibodies ch07 and ch08 have different binding epitopes than the two commercial antibodies.

This result was confirmed by a BiaCore experiment. About 100RU of hIL-13-Rα2 was immobilized on CM5 chip using amine coupling chemistry. ch07 (100nM and 200nM) and ch08 (100nM and 200nM) were sequentially injected over hIL-13-Rα2 experiment channel and control channel at flow rate 10ul/min for 150s. 50RU was reached when 100nM ch07 was injected to immobilized hIL-13Rα2. No further RU increased when ch07 concentration was increased to 200nM, indicating that the binding sites on hIL-13-Rα2 for ch07 were saturated. With the injection of 100nM ch08, 100RU was added. This positive binding signal indicates that the two antibodies lack competition. The results further confirm that antibodies ch07 and ch08 have different binding epitopes (FIG 2C).

Example 5

ch07 and ch08 Neutralization Studies

In order to assess whether ch07 and ch08 can neutralize IL-13 function, a competition ELISA was performed. An anti-Flag Ab was coated onto 96-well plates and incubated overnight at 4°C. Plates were then blocked and washed following standard

ELISA protocol. A 3-fold serial dilution of mouse antibodies, chimeric antibodies, positive control naked IL-13Rα2, and negative control hIL-21R were incubated with a constant amount of biotinylated IL-13Rα2-Fc (4x ED50) and a constant amount of IL-13 (4x ED50) at RT for 1h. 100ul of the complex was added to the ELISA plate and incubated for 1 hour at room temperature. The amount of biotin-IL-13-Rα2 bound was detected by HRP conjugated streptavidin at 1:5000 for 1 hour. The results are shown in FIG 3. Both antibodies 07 and 08 (murine and chimeric forms) do not compete with IL-13 for the IL-13-Rα2 binding site while naked IL-13-Rα2 competes with biotinylated IL-13Rα2. This indicates that antibodies 07 and 08 (murine and chimeric forms) are non-neutralizing antibodies.

Example 6

Humanization of mu08

Monoclonal murine antibody mu08 (Seq ID NOs: 23 and 24) was humanized utilizing DP-54 and DPK9 as human acceptor frameworks. Humanized 08 antibodies (hu08) were prepared by CDR grafting with or without back mutations. The CDRs of the murine mu08 antibody were identified using the Kabat scheme.

A hu08 heavy chain variable region (VH version 1.0) was constructed by directly grafting the CDRs of mu08 onto a human DP-54 framework region. The version 1.1 was made by back mutations of frame work DP-54 at positions A40T, G42D, G44R and N83S. Both v1.0 and v1.1 were cloned into pSMED2 vector containing the hIgG1 constant region. The nucleotide sequences encoding humanized hu08 heavy chain variable regions are SEQ ID: NO: 17 for v1.0 and SEQ ID: NO: 20 for v1.1. The amino sequences encoding hu08 heavy chain variable regions are SEQ ID: NO: 1 for v1.0 and SEQ ID: NO: 19 for v1.1.

A hu08 light chain variable region (VK version 1.0) was constructed by directly grafting the CDRs of murine mu08 onto a human DPK9 framework region. The version 1.1 was made by back mutations of frame work DPK9 at positions K39I, S60D, T72S, T73F and T74I. Both versions v1.0 and v1.1 were cloned into pSMEN3 vector containing the hIgKappa constant region. The nucleotide sequences encoding hu08

light chain variable regions are SEQ ID: NO: 18 for v1.0 and SEQ ID: NO: 22 for v1.1. The amino acid sequences encoding hu08 light chain variable regions are SEQ ID: NO: 5 for v1.0 and SEQ ID: NO: 21 for v1.1.

Example 7

Characterization of Humanized hu08

Humanized hu08 binding to recombinant hIL-13-Rα2 was evaluated by a standard direct ELISA. Recombinant hIL-13-Rα2 was coated onto a 96-well plate. Serially diluted chimeric antibodies or humanized antibodies in combinations of heavy and light chains of versions 1.0 and 1.1 *e.g.* hu08 v1.0 HC/1.0 LC, hu08 v1.0 HC/v1.1 LC, hu08 v1.1 HC/v1.0 LC, and hu08 v1.1 HC/v1.1 LC, were added to the plate and incubated at room temperature for 1-2 hours. The binding was detected by HRP conjugated goat anti-human Ig Kappa. The results are shown in Table 6 below and demonstrate that all four combinations of humanized antibodies are able to bind to recombinant hIL-13-Rα2 and the ED50 is comparable to ch08.

Standard FACS (Fluorescent Activated Cell Sorter) analysis was performed to assess the binding activity of the antibodies to cell surface IL-13Rα2 . A375 cells were washed with ice-cold PBS containing 1% bovine serum albumin and 0.001% sodium azide. Cells were incubated with serial dilutions of antibodies hu08 v1.0 and hu08 v1.1 *e.g.* hu08 v1.0 HC/1.0 LC, hu08 v1.0 HC/v1.1 LC, hu08 v1.1 HC/v1.0 LC, and hu08 v1.1 HC/v1.1 LC, for 30 min at 4°C and then stained with phosphatidylethanolamine-labeled goat anti-human IgG, fixed in PBS containing 4% paraformaldehyde, and analyzed on a FACScan® (BD Biosciences). The data is consistent with the binding to recombinant receptor and illustrates that the binding to cell surface antigen for all 4 combinations is comparable to ch08 (Table 6).

A competition ELISA was performed to assess whether hu08 1.0 and hu08 v1.1 compete with ch08 for IL-13-Rα2 binding. Recombinant hIL-13-Rα2 was coated onto 96-well plates at 50ul of 2µg/ml in PBS overnight at 4°C. Plates were then blocked and washed following standard ELISA protocol. A 3-fold serially diluted ch08, hu08 1.0 and hu08 v1.1, and negative control ch07 (2x final concentration) were mixed with

biotinylated ch08 (2x EC_{50}). 50μl of the antibody and biotin-ch08 mixture were added to the plate and incubated for 1 hour at room temperature. The amount of biotin-ch08 bound was detected by HRP conjugated streptavidin. The results are shown in Table 6. All 4 combinations of humanized antibodies are similar to chimeric antibody ch08 in competing with biotinylated ch08, indicating that hu08 1.0 and hu08 v1.1 retained the same binding epitope as ch08 and have similar affinity to soluble and cell surface IL-13-Rα2.

Table 6

Antibody	EC50 (nM) rec hIL-13Rα2	EC50 (nM) A375 cells	IC50 (nM) Biotin-ch08
ch08Hc+Lc	0.152	5.637	1.979
hu08 v1.0/1.0	0.175	4.098	2.120
hu08 v1.0/1.1	0.143	6.522	2.144
hu08 v1.1/1.0	0.163	4.152	2.562
hu08 v1.1/1.1	0.198	5.157	3.164

hu08 v1.0 HC/v1.0 LC and hu08 v1.1HC /v1.1LC were scaled up to generate purified proteins. Both antibodies were transiently expressed into HEK293F suspension cells. Surprisingly, humanization of 08 has improved the antibody production yield by 5-6 fold compared to the yield of ch08 (Table 7).

Table 7

Antibody name	Expression level (ug/ml)
ch08	15
hu08v1.0/1.0	89.6
hu08v1.1/1.1	72.3

There is a direct correlation between the thermal stability of a protein or protein domain with the overall stability of the protein or protein domain. A higher melting point of a protein or protein domain often provides improved manufacturability and longer shelf life/stability. Thermal stability of ch08 and hu08 (v 1.0 and v1.1) was examined by

Differential Scanning Calorimetry (DSC). Thermal unfolding of chimeric and humanized antibodies by DSC was performed using a standard protocol on a MicroCal VP-DSC instrument. Both of the humanized antibodies, hu08 v1.0/1.0 and hu08 v1.1/1.1, show a higher Tm2 (Fab) than the chimeric version ch08 (Table 8). This demonstrates that humanization of cu08 improves the thermal stability of this antibody.

Table 8

	CH2	Fab	CH3
Antibody	Tm1 (^{0}C)	Tm2 (^{0}C)	Tm3 (^{0}C)
ch08	73.41+0.47	70.44+0.04	84.35+0.09
hu08v1.0/1.0	73.48±0.19	80.29±0.02	85.48±0.14
hu08v1.1/1.1	71.12+0.13	80.23+0.02	

The binding kinetics of hu08 antibodies was conducted on a Biacore® T100 as described above with the results shown in Table 9

Table 9

Antibody	Antigen	pH	ka (1/Ms)on	kd (1/s)off	KD (nM)
hu08 v1.0	hIL-13Rα2	7.4	9.75E+04	2.46E-04	2.52E-09
hu08 v1.0	hIL-13Rα2	6.0	5.29E+04	2.34E-04	4/42E-09

Example 8

Humanization of mu07

The general strategy of humanizing monoclonal murine antibody mu07 is the same as described for mu08 in Example 6. A humanized hu07 heavy chain variable region (VH version 1.0) was constructed by directly grafting the CDRs of mu07 onto a human DP-54 framework region. The versions 1.1-1.5 were made by back mutations of frame work DP-54 at various positions (Table 10). All versions were cloned into pSMED2 vector containing hIgG1 constant region.

A hu07 light chain variable region (VK version 1.0) was constructed by directly grafting the CDRs of mu07 onto a human DPK9 framework region. The versions 1.1-1.7 were made by back mutations of frame work DPK9 at various positions (Table 10). All versions were cloned into pSMEN3 vector containing hIg Kappa constant region. SEQ ID numbers of the amino acid sequences encoding hu07 variable regions are listed in Table 10.

Table 10:

	hu07 VH		hu07 VK		
VH Variants	aa SEQ ID	Back-mutation	VL Variants	aa SEQ ID	Back-mutation
hu07 VH v1.0	9		hu07 VK v1.0	13	
hu07 VH v1.1	37	T28S, F29L, A49G, F67L, N76S	hu07 VK v1.1	42	K41S, A42S, D70S
hu07 VH v1.2	38	R71K	hu07 VK v1.2	43	L47W
hu07 VH v1.3	39	T28S, F29S, R71K	hu07 VK v1.3	44	F71Y
hu07 VH v1.4	40	T28S, F29S,	hu07 VK v1.4	45	L47W, F71Y
hu07 VH v1.5	41	T28S, F29S, A49G, R71K	hu07 VK v1.5	46	K41S, A42S, D70S, L47W
			hu07 VK v1.6	47	K41S, A42S, D70S, F71Y
			hu07 VK v1.7	48	K41S, A42S, D70S, L47W, F71Y

Example 9

Characterization of Humanized hu07

To evaluate the binding/competition properties of the various versions of hu0, transient transfections with 19 combinations of hu07 heavy and light chains were performed in COS-1 M6 cells. 6 heavy chains and 3 light chains were included: chimeric heavy chain, humanized v1.0-1.5 heavy chain, chimeric light chain and humanized v1.0-1.1 light chain. Conditioned media (CM) was harvested 2 days after transfection and subjected for direct binding to recombinant IL-13-Rα2 by standard ELISA, cell surface receptor binding by cell-based ELISA using A375 cells, and competition ELISA with biotinylated ch07, utilizing protocols known in the art.

Based on initial screening data from CM, heavy chain v1.5 was selected for further study. Heavy chain v1.5 was paired with chimeric, humanized v1.0 and v1.1 light chain. Table 11 summarizes the binding activity, competition properties and cytoxicity of hu07 antibodies. hu07 v1.5 paired with chimeric light chain Kc demonstrates a similar ED50 and IC50 to the chimeric antibody. The competition activity on the A375 cells and recombinant protein were decreased when this heavy chain v1.5 was paired with light chain v1.0 and v1.1.

Table 11

	IC50 (nM) Competition ELISA Plate	ED50 (nM) ELISA rhIL-13Rα2	ED50 (nM) cELISA A375 cells	IC50 (nM) Competition ELISA A375 cells	IC50 (nM) Saporin Assay A375 cells
ch07	2.21	0.217	0.85	17.27	0.08
hu07v1.5/kc	3.03	0.183	0.87	7.67	ND
hu07v1.5/v1.0	62.00	0.754	1.17	12.27	0.19
hu07v1.5/v1.1	46.62	1.214	1.29	19.00	0.20

hu07 heavy chain v1.5 was used for light chain optimization. Transient transfections with 10 combinations of hu07 heavy and light chains were performed in COS-1 M6 cells. The heavy chain v1.5 was paired with chimeric light chain and humanized versions v1.0-1.7. CM was harvested and used in experiments to determine binding affinity to recombinant hIL-13α2 (rhIL-13α2) by ELISA and competition activity to biotinylated ch07 by competition ELISA. The data indicates that the combination of heavy chain v1.5 and light chain v1.7 is optimal. This result was confirmed with purified protein (Table 12).

Table 12

	ED50 (nM) rhIL-13Rα2	**IC50 (nM) Competition ELISA Plate**
ch07	0.39	2.21
hu07Hv1.5/kv1.7	0.39	7.7

Example 10

Species cross reactivity of ch07, hu07, ch08 and hu08

Cynomolgous (cyno) monkey IL-13-Rα2 (extracellular domain (ECD)) and transmembrane domain (TM) were isolated from cyno monkey testis and adipose tissues by RT-PCR. The amino acid sequence of cyno IL-13-Rα2 is shown in SEQ ID NO: 27. The identity is 94% between human and cyno IL-13Rα2.

The ECD/TM domain of cyno IL-13-Rα2 fused with Flag tag at the C-terminal end was cloned into the pSMED2 expression vector. HEK293 suspension cells were transiently transfected with cyno-IL-13-Rα2 containing plasmid and pSMED2 vector (as a mock transfection). The cells were harvested 72 hours later and subjected to FACS analysis. 4 antibodies including ch07, ch08, hu08v1.0/1.0 and hu08v1.1/1.1were tested. The binding on the cell surface cyno-IL-13-Rα2 was detected with R-Phycoerythrin-labeled goat anti-human or mouse IgG. The data demonstrates that

ch07, ch08, hu08v1.0/1.0 and hu08v1.1/1.1 are able to bind to the cell surface cyno-IL-13-Rα2 and have similar binding affinities (ED50) (Table 13).

Table 13

Antibody	ED50 (nM)
ch07	1.494
ch08	1.814
hu08v1.0/1.0	1.961
hu08v1.1/1.1	2.141

The binding of hu07 and hu08 to mouse IL-13-Rα2 was evaluated by direct ELISA. The identity between human and mouse IL-13-Rα2 at the amino acid level is approximately 64%. Recombinant mIL-13-Rα2 or hIL-13-Rα2 (as positive control) was coated onto a 96-well plate. Purified chimeric ch07 and ch08 were serially diluted and added to the antigen coated plate. The bound antibodies were detected by HRP conjugated goat anti-human IgGFc specific secondary antibody. There was no detectable signal for mIL-13-Rα2 binding while the binding to the positive control hIL-13-Rα2 was strong, indicating that hu07 and hu08 do not cross-react to murine mIL-13Rα2.

Example 11

Binding to Human Cell Lines Expressing IL13R-α2

Cell lines expressing the IL-13-Rα2 antigen and the negative control cells were plated at a density of 200,000 cells per well of 96 deep well plates and kept on ice. The mouse monoclonal antibodies mu07 or mu08 prepared in 3% bovine serum albumin BSA in Dulbecco's phosphate buffered saline (DPBS) were added to the plate at a final concentration of 10 µg/mL. The plates were then incubated on ice for 1 hour followed by 2 washes. The secondary antibody, PE (phycoerythrin) conjugated goat anti-mouse

IgG Fc was added to the wells. After 30 minutes of incubation at 4°C, the mean fluorescence intensity was then analyzed by FACS on a FACScan™ (BD Biosciences).

The data in Table 14 indicates that the mu07 and mu08 antibodies bind to a diverse panel of IL-13R-α2 positive cell lines from various disease indications.

Table 14

Human Cell Line	Mean Fluorescent Intensity		IL-13-Rα2 Expression
	mu07	mu08	
PC3MM2 (prostate)	84000	72000	3+
U87MG (glioblastoma)	61000	62000	3+
A375 (melanoma)	53000	46000	3+
H460 (lung, cisplatin resistant)	28000	22000	2+
Hs766T (pancreatic)	13000	14000	2+
A498 (renal)	13000	5000	1+
SW626 (ovarian)	9000	8000	1+
H460 (lung)	800	300	0

Example 12

Internalization

Antibody internalization is a critical characteristic for delivering ADCs for cytotoxicity in IL-13-Rα2 expressing cells. Internalization of the antibody after binding to IL-13-Rα2 was examined using mu07 and mu08 antibodies and a positive control mouse monoclonal antibody (ab27414), on four cell lines (PC3MM2, A375, Hs766T, and H460R). The antibodies (10 µg/mL) were incubated with the various cells for 1 hour on ice and unbound antibody was removed by washing twice with cold media. The cell culture plates were incubated at 37°C. Samples of the cells were fixed at 15 minutes

and at 4 hours. The percent internalization at different timepoints is shown in Table 15. The data show that mu07 and mu08 are readily internalized into IL-13-Rα2 expressing cells.

Table 15

% internalization								
Primary Abs	15 min				4 hours			
	A375	Hs766T	PC3MM2	H460R	A375	Hs766T	PC3MM2	H460R
mu07	63.1	69.6	88.6	74.4	38.1	69.0	50.3	38.4
mu08	68.0	64.1	81.9	55.9	61.8	76.3	41.6	47.4
ab27414	49.3	60.8	60.2	57.5	68.2	73.6	49.7	49.2

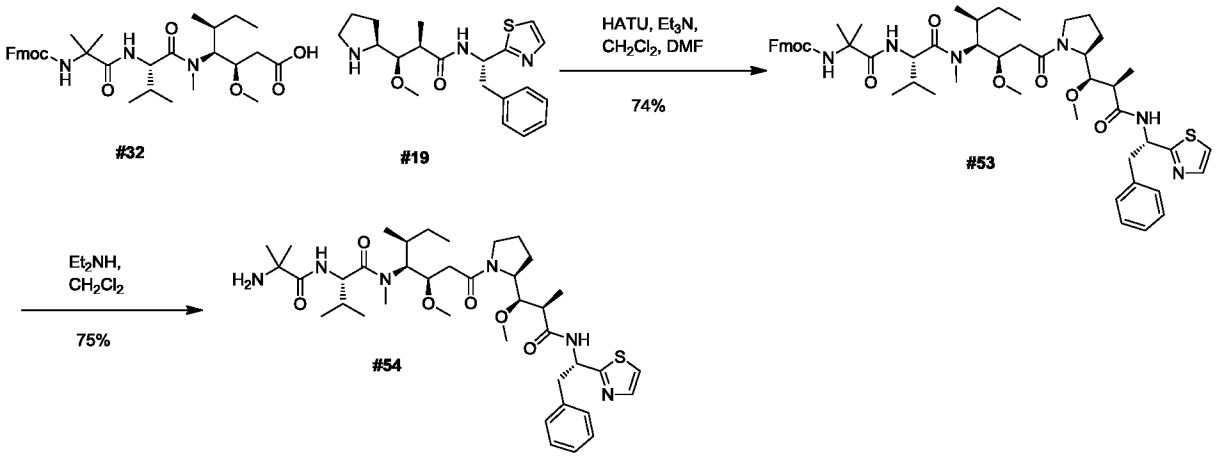
Example 13

Synthesis of compounds 0101 and 3377

Compounds 0101 and 3377 were prepared according to the methods described in US Patent Application No.13/670,612, herein incorporated by reference.

Experimental for compound 0101 (#54 in the schematic)

Preparation of 2-Methylalanyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(1S)-2-phenyl-1-(1,3-thiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide (#54)



Step 1. Synthesis of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-2-methylalanyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*S*)-2-phenyl-1-(1,3-thiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (#53). According to general procedure D, from #32 (2.05 g, 2.83 mmol, 1 eq.) in dichloromethane (20 mL, 0.1 M) and *N,N*-dimethylformamide (3 mL), the amine #19 (2.5 g, 3.4 mmol, 1.2 eq.), HATU (1.29 g, 3.38 mmol, 1.2 eq.) and triethylamine (1.57 mL, 11.3 mmol, 4 eq.) was synthesized the crude desired material, which was purified by silica gel chromatography (Gradient: 0% to 55% acetone in heptane), producing #53 (2.42 g, 74%) as a solid. LC-MS: *m/z* 965.7 [M+H$^+$], 987.6 [M+Na$^+$], retention time = 1.04 minutes; HPLC (Protocol A): *m/z* 965.4 [M+H$^+$], retention time = 11.344 minutes (purity > 97%); ^{1}H NMR (400 MHz, DMSO-d_6), presumed to be a mixture of rotamers, characteristic signals: δ 7.86-7.91 (m, 2H), [7.77 (d, *J*=3.3 Hz) and 7.79 (d, *J*=3.2 Hz), total 1H], 7.67-7.74 (m, 2H), [7.63 (d, *J*=3.2 Hz) and 7.65 (d, *J*=3.2 Hz), total 1H], 7.38-7.44 (m, 2H), 7.30-7.36 (m, 2H), 7.11-7.30 (m, 5H), [5.39 (ddd, *J*=11.4, 8.4, 4.1 Hz) and 5.52 (ddd, *J*=11.7, 8.8, 4.2 Hz), total 1H], [4.49 (dd, *J*=8.6, 7.6 Hz) and 4.59 (dd, *J*=8.6, 6.8 Hz), total 1H], 3.13, 3.17, 3.18 and 3.24 (4 s, total 6H), 2.90 and 3.00 (2 br s, total 3H), 1.31 and 1.36 (2 br s, total 6H), [1.05 (d, *J*=6.7 Hz) and 1.09 (d, *J*=6.7 Hz), total 3H].

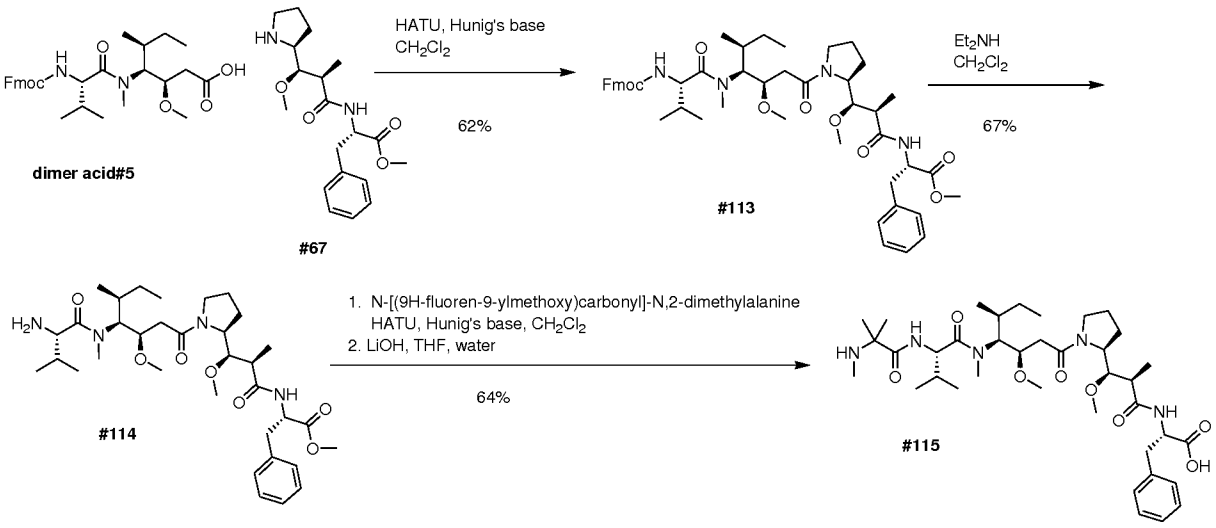
Step 2. Synthesis of 2-methylalanyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*S*)-2-phenyl-1-(1,3-thiazol-2-

yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (#54)

According to general procedure A, from #53 (701 mg, 0.726 mmol) in dichloromethane (10 mL, 0.07 M) was synthesized the crude desired material, which was purified by silica gel chromatography (Gradient: 0% to 10% methanol in dichloromethane). The residue was diluted with diethyl ether and heptane and was concentrated *in vacuo* to afford #54 (406 mg, 75%) as a white solid. LC-MS: *m/z* 743.6 [M+H$^+$], retention time = 0.70 minutes; HPLC (Protocol A): *m/z* 743.4 [M+H$^+$], retention time = 6.903 minutes, (purity > 97%); ^{1}H NMR (400 MHz, DMSO-d_6), presumed to be a mixture of rotamers, characteristic signals: δ [8.64 (br d, *J*=8.5 Hz) and 8.86 (br d, *J*=8.7 Hz), total 1H], [8.04 (br d, *J*=9.3 Hz) and 8.08 (br d, *J*=9.3 Hz), total 1H], [7.77 (d, *J*=3.3 Hz) and 7.80 (d, *J*=3.2 Hz), total 1H], [7.63 (d, *J*=3.3 Hz) and 7.66 (d, *J*=3.2 Hz), total 1H], 7.13-7.31 (m, 5H), [5.39 (ddd, *J*=11, 8.5, 4 Hz) and 5.53 (ddd, *J*=12, 9, 4 Hz), total 1H], [4.49 (dd, *J*=9, 8 Hz) and 4.60 (dd, *J*=9, 7 Hz), total 1H], 3.16, 3.20, 3.21 and 3.25 (4 s, total 6H), 2.93 and 3.02 (2 br s, total 3H), 1.21 (s, 3H), 1.13 and 1.13 (2 s, total 3H), [1.05 (d, *J*=6.7 Hz) and 1.10 (d, *J*=6.7 Hz), total 3H], 0.73-0.80 (m, 3H).

Experimental for compound 3377 (#115 in the schematic)

Preparation of N,2-dimethylalanyl-N-{(1S,2R)-4-{(2S)-2-[(1R,2R)-3-{[(1S)-1-carboxy-2-phenylethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-2-methoxy-1-[(1S)-1-methylpropyl]-4-oxobutyl}-N-methyl-L-valinamide, trifluoroacetic acid salt (#115).



Step 1. Synthesis of methyl N-{(2R,3R)-3-[(2S)-1-{(3R,4S,5S)-4-[{N-[(9H-fluoren-9-ylmethoxy)carbonyl]-L-valyl}(methyl)amino]-3-methoxy-5-methylheptanoyl}pyrrolidin-2-yl]-3-methoxy-2-methylpropanoyl}-L-phenylalaninate (#113). To a stirring mixture of dimer acid#5 (12. 1g, 23.0 mM) and #67 (11.5 g, 23.0 mM) in 75 mL of dichloromethane under nitrogen, HATU (10.8 g, 27.6 mM) was added followed by Hunig's base (12.1 mL, 69.0 mM). The reaction was allowed to stir at room temperature for 15 hours. Reaction was concentrated to a smaller volume, taken up with ethyl acetate and washed with 1 N HCl two times. The organic layer was then washed with brine, dried over sodium sulfate, filtered, and concentrated *in vacuo*. Residue was then purified by silica gel chromatography (Gradient: 0% to 70% acetone in heptanes), producing #113 (12.3 g, 62%) as a white solid. LC-MS (Protocol Q): *m/z* 855.3 [M+H$^+$], 877.2 [M+Na$^+$], retention time = 2.32 minutes; HPLC (Protocol R): */z* 855.5 [M+H$^+$], retention time = 9.596 minutes (purity > 97%).

Step 2. Synthesis of methyl N-{(2R,3R)-3-methoxy-3-[(2S)-1-{(3R,4S,5S)-3-methoxy-5-methyl-4-[methyl(L-valyl)amino]heptanoyl}pyrrolidin-2-yl]-2-methylpropanoyl}-L-phenylalaninate (#114). According to general procedure A, from #113 (12 g, 14 mmol, 1 eq.), dichloromethane (60 mL, 0.24 M) and diethylamine (40 mL, 390 mM) was synthesized #114 (5.9 g, 67%) white/slight yellow solid after purification by silica gel chromatography (Gradient: 0% to 25% methanol in dichloromethane). LC-MS (Protocal Q): *m/z* 633.0 [M+H$^+$], retention time = 1.19

minutes. HPLC (Protocol A): *m/z* 633.5 [M+H$^+$], retention time = 7.142 minutes (purity > 98%).

Step 3. Synthesis of N,2-dimethylalanyl-N-{(1S,2R)-4-{(2S)-2-[(1R,2R)-3-{[(1S)-1-carboxy-2-phenylethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-2-methoxy-1-[(1S)-1-methylpropyl]-4-oxobutyl}-N-methyl-L-valinamide-trifluoroacetic acid salt (#115). To a stirring mixture of N-[(9H-fluoren-9-ylmethoxy)carbonyl]-N,2-dimethylalanine (167 mg, 0.493 mM), #114 (260 mg, 0.411 mM), and HATU (188 mg, 0.493 mM) in 10 mL of dichloromethane, Hunig's base (0.14 mL, 0.82 mM) was added. The reaction was allowed to stir at room temperature for 1 hour and 20 minutes. Reaction was reduced down. THF (9 mL) was added to crude material and to this stirring mixture lithium hydroxide (49.2 mg, 2.06 mM) dissolved in 3 mL of water was added. The reaction was allowed to stir at room temperature for 4 hours. Reaction was concentrated down followed by purification by medium pressure reverse phase C18 chromatography (Gradient: 5% to 45% water in acetonitrile with 0.02% TFA in each phase) #115 (218 mg, 64%) white solid. LC-MS (Protocol Q): *m/z* 718.7 [M+H$^+$], 740.6 [M+Na$^+$], retention time = 1.21 minutes. HPLC (Protocol A at 45 °C): *m/z* 718.4 [M+H$^+$], retention time = 6.903 minutes.

Example 14

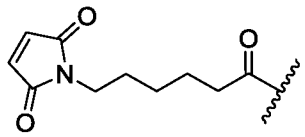
Preparation of anti-IL-13-Rα2 ADCs

The ADCs of the present invention can be prepared using a section of the linker having a reactive site for binding to a chemical compound and introducing another section of the linker having a reactive site for an antibody. In one aspect, a linker has a reactive site which has an electrophilic group that is reactive with a nucleophilic group present on an antibody unit, such as an antibody. Useful nucleophilic groups on an antibody include but are not limited to, sulfhydryl, hydroxyl and amino groups. The heteroatom of the nucleophilic group of an antibody is reactive to an electrophilic group on a linker and forms a covalent bond to a linker. Useful electrophilic groups include, but are not limited to, maleimide and haloacetamide groups.

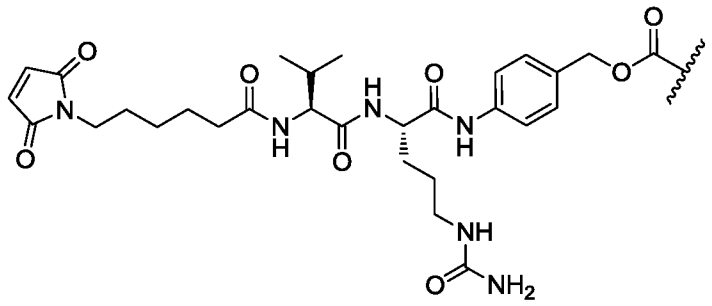
A linker has a reactive site which has a nucleophilic group that is reactive with an

electrophilic group present on an antibody unit. The electrophilic group on an antibody provides a convenient site for attachment to a linker. Useful electrophilic groups on an antibody include, but are not limited to, aldehyde and ketone carbonyl groups. The heteroatom of a nucleophilic group of a linker can react with an electrophilic group on an antibody and form a covalent bond to the antibody. Useful nucleophilic groups on a linker include, but are not limited to, hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide.

As used herein, “mc-” refers to:.



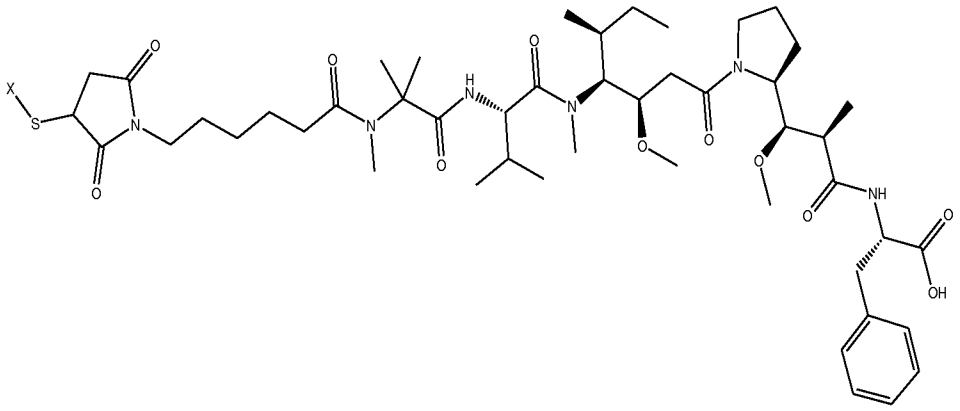
As used herein, “vc-” refers to:



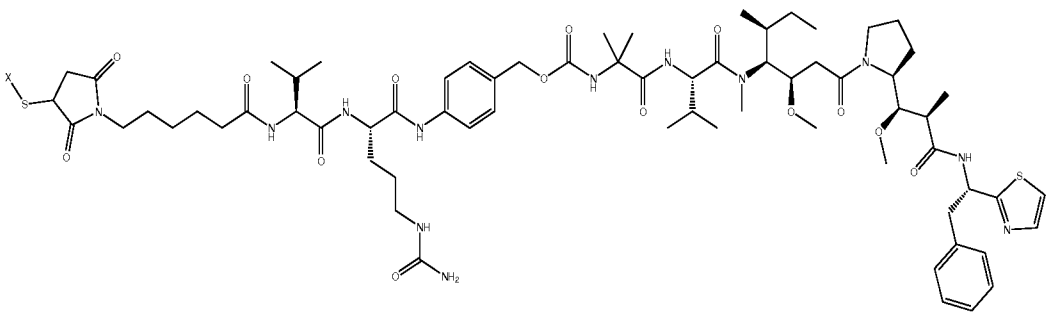
. The anti-IL-13-Rα2 ADCs were prepared via partial reduction of the mAb with tris(2-carboxyethyl)phosphine (TCEP) followed by reaction of reduced cysteine residues with the desired maleimide terminated linker-payload. In particular, hu08 was partially reduced via addition of 2.2 molar excess of tris(2-carboxyethyl)phosphine (TCEP) in 100 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer), pH 7.0 and 1 mM diethylenetriaminepentaacetic acid (DTPA) for 2 h at 37 °C. The desired linker-payload was then added to the reaction mixture at a linker-payload/mAb molar ratio of 7.0 maleimidocapronic – valine-citruline-*p*-aminobenzyloxycarbonyl- aurstatin-0101 [vc-

0101, see below]), or 7.0 maleimidocapronic-auristatin-3377 [mc-3377, see below] and reacted for an additional 1 h at 25°C in the presence of 15% v/v of dimethylacetamide (DMA). After the 1 h incubation period, N-ethylmaleimide (3 fold excess for vc-0101 and for mc-3377) was added to cap the unreacted thiols and is allowed to react for 15 minutes, followed by addition of 6 fold excess L-Cys to quench any unreacted linker-payload. The reaction mixture was dialyzed overnight at 4 °C in phosphate buffered saline (PBS), pH 7.4, and purified via SEC (AKTA explorer, Superdex 200 10/30 GL column). The ADC was further characterized via size exclusion chromatography (SEC) for purity, hydrophobic interaction chromatography (HIC), and liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI MS) to calculate drug-antibody ratio (loading). The protein concentration was determined via UV spectrophotometer.

mc-3377 (conjugation to antibody X through a cysteine residue)



vc-0101 (conjugation to antibody X through a cysteine residue)



Example 15

In Vitro Cytotoxicity Assay

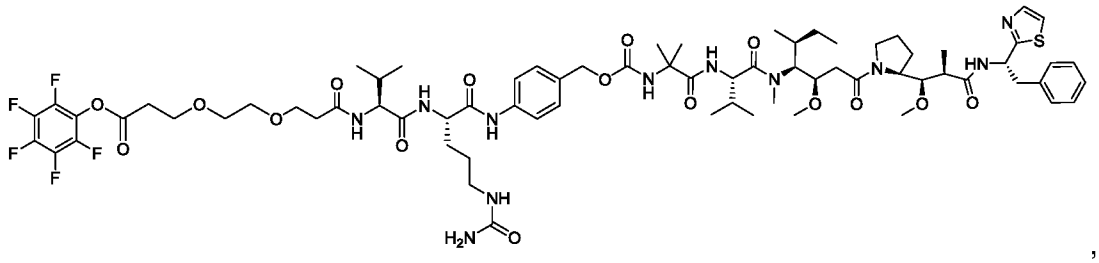
Cell lines expressing the IL-13-Rα2 antigen and a negative control cell line, were cultured with increasing concentrations of anti-IL-13-Rα2 ADC. After four days, viability of each culture is assessed. IC_{50} values were calculated by logistic non-linear regression and are presented as ng Ab/mL. The preferred linker payloads were vc-0101 and mc-3377.

Humanized anti-IL-13Rα2 antibody hu08 was conjugated to various linker-payload combinations as provided in Table 16. The antibody drug conjugates were prepared according to the methods described in US Patent Application No.13/670,612, which is incorporated herein by reference, with the corresponding nomenclature and experimental chemical synthesis schematic number indicated in Table 16.

Table 16

ADC Linker-Payload	Corresponding ADC Linker-Payload #
hu08-vc-0101	IL13Rα2 -AB08-v1010-hG1-(C)_mcValCitPABC-#54
hu08-mc-3377	IL13Rα2 -AB08-v1010-hG1-(C)_mc-#115
hu08-mc-0131	IL13Rα2 -AB08-v1010-hG1-(C)_mc-0#118
hu08-Malpeg-6121	IL13Rα2 -AB08-v1010-hG1-(C)_MalPeg6C2-#117
hu08-Malpeg-0131	IL13Rα2 -AB08-v1010-hG1-(C)_Mal(H2O)Peg6C2-0#118
hu08-mc-6121	IL13Rα2 -AB08-v1010-hG1-(C)_mc-#117
hu08-vc-3906	IL13Rα2 -AB08-v1010-hG1-(C)_mcValCitPABC-#226
hu08-vc-6780	IL13Rα2 -AB08-v1010-hG1-(C)_mcValCitPABC-#112
hu08-mc-8261	IL13Rα2 -AB08-v1010-hG1-(C)_mc-#69
hu08-mc-3906	IL13Rα2 -AB08-v1010-hG1-(C)_mc-#226
hu08-MalPeg-8261	IL13Rα2 -AB08-v1010-hG1-(C)_MalPeg6C2-#69
huIgG8.8-vc-0101	huIgG8.84-mcValCitPABC-#54
huIgG8.8-mc-3377	huIgG8.84-mc-#115

Further, a mutant version of hu08 (hu08MAC) was generated as described in Example 21 and according to standard protocols. Compound 0101 was conjugated to a cleavable linker to form the structure:



,

and thereafter conjugated to hu08MAC according to the techniques described herein to form hu08MAC -0101.

The data demonstrates that the anti-IL-13-Rα2 antibody hu08v1.0/1.0 conjugated to six different auristatin payloads was effective against both of the IL-13-Rα2 positive cell lines tested (PC3MM2 and A375), having an IC_{50} ranging from 1.1 to 4.9 ng Ab/mL or 7.3-32.7 pM) (Table 17). Further, hu08MAC-0101 was effective against both of the IL-13Rα2 positive cell lines tested (PC3MM2 and A375), having an IC_{50} of 7.9 ng Ab/mL. All ADCs were not active against the IL-13-Rα2 negative cell line, H460, and the non- IL-13-Rα2 binding control ADCs, hIgG8.8-vc-0101 and hIgG8.8-mc-3377, were not active against any of the cell lines tested.

Table 17

ADC	DAR	IC_{50} (ng Ab/mL)		
		PC3MM2	A375	H460
hu08-vc-0101	3.2	2.5	3.8	>400000
hu08-mc-3377	4.3	1.2	2.2	>400000
hu08-mc-0131	3.2	1.3	2.1	>400000
hu08-MalPeg-6121	3.3	3.5	3.4	>400000
hu08-MalPeg-0131	2.9	2.9	4.9	>400000
hu08-mc-6121	3.3	1.1	2.4	>400000
hu08-mc-3906	3	1.5	2.9	>400000
hu08 vc-6780	4	1.2	2.2	>400000
hu08MAC-0101	1.9	4.9	7.9	>400000
hIgG8.8-vc-0101	3.7	>400000	>400000	>400000
hIgG8.8-mc-3377	4.3	>400000	>400000	>400000

Example 16

Subcutaneous Xenograft Models

Female, athymic (nude) mice were injected s.c. with PC3MM2 or A375 tumor cells. Mice with staged tumors, approximately 0.2 to 0.8 g (n = 8 to 10 mice/treatment group), were administered intravenously q4d x 4 with normal saline (vehicle), hu08v1.0/1.0 ADCs with linker-payloads vc-0101, vc-6780, vc-3906, mc-8261, mc-0131, mc-6121, mc-3377, MalPeg-8261, MalPeg-0131, MalPeg-6121, and MalPeg-3906, and a non-binding Ab (hIgG8.8) conjugated with vc-0101 or mc-3377, at a dose of 2 or 3 mg Ab/kg. The ADCs were dosed based on Ab content. Tumors were measured at least once a week and their size is calculated as $mm^3 = 0.5 \times (\text{tumor width}^2) \times (\text{tumor length})$.

The in vivo efficacy results listed in Table 18 show a range of anti-tumor activity with the various ADCs tested. The relative order of potency is hu08-vc-0101 > hu08-vc-6780 ≥ hu08-mc-0131 > hu08-mc-6121 > hu08-mc-3906 > hu08-MalPeg-0131 >hu08-MalPeg-6121 > hu08-MalPeg-3906 > hu08-mc-8261.

The data in Table 19 indicates that ADCs hu08-vc-0101 and hu08-mc-3377 were efficacious 3mg/kg in reducing tumor growth in the PC3MM2. Compared to the vehicle control group which was terminated at Day 15 due to large size of tumors (>2500 mm^3 from some of animals), hu08-vc-0101 and hu07-mc-3377 have 5 out of 8 or 3 out of 8 animals without measurable tumors at Day 76, respectively. Similar to the vehicle control group, irrelevant ADC control groups of hIgG8.8-vc-0101 at 3 mg/kg and hIgG8.4-mc-3377 at 10 mg/kg was terminated at Day 15 and Day 19 respectively due to the large size of tumors in the groups, respectively. These results demonstrate that the significant therapeutic efficacy (some animals were cured of disease) from hu08-vc-0101 and hu07-mc-3377 was IL-13-Rα2 target-mediated.

Table 18

ADC	Dose (mg/kg) Q4dx4	PC3MM2 xenograft, tumor volume (mm^3 +/- SEM)							
		Day -1	Day 3	Day 8	Day 16	Day 20	Day 30	Day 42	Day 52
Vehicle	0	638 ± 27	1149 ± 82	1707 ± 133	GT	GT	GT	GT	GT
hu08-Malpeg-3906	2	642 ± 36	1036 ± 60	1176 ± 51	GT	GT	GT	GT	GT
hu08-mc-8261	2	642 ± 51	1088 ± 121	1429 ± 158	GT	GT	GT	GT	GT
hu08-mc-0131	2	637 ± 44	1004 ± 73	778 ± 83	GT	GT	GT	GT	GT
hu08-Malpeg-6121	2	638 ± 36	947 ± 85	1000 ± 126	693 ± 129	780 ± 198	GT	GT	GT
hu08-Malpeg-0131	2	649 ± 39	1085 ± 54	1040 ± 88	GT	GT	GT	GT	GT
hu08-vc-0101	2	646 ± 36	899 ± 54	557 ± 49	243 ± 28	201 ± 20	113 ± 17	207 ± 49	532 ± 151
hu08-vc-6780	2	641 ± 28	850 ± 100	652 ± 54	279 ± 55	217 ± 45	230 ± 133	GT	GT
hu08-mc-6121	2	636 ± 37	909 ± 63	821 ± 93	441 ± 83	414 ± 104	GT	GT	GT
hu08-mc-3906	2	637 ± 26	875 ± 48	806 ± 70	611 ± 150	GT	GT	GT	GT
hu08-Malpeg-8261	2	645 ± 34	991 ± 71	1220 ± 115	GT	GT	GT	GT	GT

GT= group terminated due to large tumor size

Table 19

ADC	Dose (mg/kg) Q4dx4	PC3MM2 xenograft, tumor volume(mm^3+/-SEM)							
		Day -1	Day 2	Day 10	Day 19	Day 40	Day 51	Day 61	Day 71
vehicle	0	340 ± 16	573 ± 47	1441 ± 176	1975 ± 272	GT	GT	GT	GT
hIgG8.84-mc-3377	10	328 ± 27	459 ± 63	295 ± 121	544 ± 258	GT	GT	GT	GT
hu08-mc-3377	3	337 ± 21	385 ± 36	41 ± 12	0 ± 0	78 ± 36	346 ± 147	616 ± 243	902 9 364
hu08-vc-0101	3	339 ± 18	433 ± 45	38 ± 14	6 ± 6	110 ± 110	230 ± 230	GT	GT
hIgG8.8-vc-0101	3	333 ± 23	449 ± 15	686 ± 134	GT	GT	GT	GT	GT

GT= group terminated due to large tumor size.

The data in Table 20a indicates that ADCs of hu08-vc-0101 and hu08 –mc-3377 were efficacious at 3 mg/kg in a second in vivo xenograft model using A375 cells. The vehicle control group and irrelevant ADC control groups of hIgG8.8-vc-0101 and hIgG8.8-mc-3377 were terminated due to large tumor size at Day 19, 22, 27, respectively. Treatment with hu08-vc-0101 and hu08-mc-3377 at 3 mg/kg caused tumor regression in all animals and provided significant survival advantage. No measureable tumor was observed in all animals treated with hu08-mc-3377 at Day 19 - 22. Although some tumors relapsed, there were 5 out of 10 animals without measurable tumors at Day 71. The group treated with hu08-vc-0101 was monitored over 100 days and 8 of animals had no measurable tumors at Day 19-100. These results demonstrate potent antitumor activities of hu08-vc-0101 and hu08-mc-3377 against IL-13-Rα2 positive tumors.

Table 20a

ADC	Dose (mg/kg) Q4dx4	A375 Xenograft Tumor Volume (mm^3 ± SEM)							
		Day -1	Day 2	Day 13	Day 19	Day 40	Day 51	Day 61	Day 71
Vehicle	0	340 ± 16	573 ± 47	1441 ± 176	1975 ± 272	GT	GT	GT	GT
hIgG8.8-mc-3377	3	328 ± 27	459 ± 63	295 ± 121	544 ± 258	GT	GT	GT	GT
Hu08-mc-3377	3	337 ± 21	385 ± 36	41 ± 14	0 ± 0	78 ± 36	346 ± 147	616 ± 243	902 ± 364
Hu08-vc-0101	3	336 ± 19	407 ± 42	43 ± 15	7 ± 15	122 ± 7	256 ± 122	0 ± 0	0 ± 0
hIgG8.8-vc-0101	3	333 ± 23	449 ± 15	686 ± 135	1145 ± 212	GT	GT	GT	GT

GT= group terminated due to large tumor size.

The data in Tables 20b and 20c indicates that ADCs hu08-vc-0101 and hu08-mc3377 were efficacious in a third in vivo xenograft model using the HEY-C2 ovarian cancer cell line. The vehicle control group and negative ADC control group's hIgG8.8-vc0101 (3 mg/kg and 10 mg/kg) and hIgG8.8-mc-3377 (10 mg/kg) were terminated due to large tumor size as indicated with the GT designation. Treatment with hu08-vc-0101 and hu08-mc-3377 at dose levels of 1, 3 and 10 mg/kg, provided a dose-dependent response. Five out of nine animals treated with 3 mg/kg of hu08-vc-0101 and seven out of nine animals treated with 10 mg/kg of hu08-vc-0101 survived to end of the study (Day 103). Similarly, five out of nine animals treated with 3 mg/kg of hu08-mc-3377 and nine out of nine animals treated with 10 mg/kg of hu08-mc-3377 survived to the end of the study (Day 103).

Table 20b

ADC	Dose (mpk) Q4d	HEY-C2 Xenograft, tumor volume (mm^3 ± SEM)											
		Day -1	Day 3	Day 12	Day 23	Day 30	Day 40	Day 51	Day 60	Day 72	Day 79	Day 95	Day 103
vehicle	0	211 ± 16	375 ± 21	919 ± 53	2311 ± 156	GT	GT	GT	GT	GT	GT	GT	GT
hAB08 mc_3377	1	211 ± 17	324 ± 23	337 ± 25	510 ± 128	665 ± 203	GT	GT	GT	GT	GT	GT	GT
	3	211 ± 17	319 ± 38	250 ± 37	207 ± 41	239 ± 64	336 ± 131	602 ± 258	GT	GT	GT	GT	GT
	10	211 ± 16	303 ± 28	181 ± 17	114 ± 10	106 ± 7	84 ± 16	69 ± 14	53 ± 14	42 ± 11	41 ± 12	121 ± 59	308 ± 166
hIgG 8.8 mc_3377	10	212 ± 18	375 ± 46	557 ± 53	650 ± 183	GT	GT	GT	GT	GT	GT	GT	GT

Table 20c

ADC	Dose (mpk) Q4d	HEY-C2 Xenograft, tumor volume (mm^3 ± SEM)											
		Day -1	Day 3	Day 12	Day 23	Day 30	Day 40	Day 51	Day 60	Day 72	Day 79	Day 95	Day 103
hAB08 vc_0101	1	211 ± 16	335 ± 27	331 ± 43	479 ± 96	697 ± 177	GT	GT	GT	GT	GT	GT	GT
	3	210 ± 18	324 ± 19	258 ± 36	200 ± 56	222 ± 90	354 ± 239	GT	GT	GT	GT	GT	GT
	10	211 ± 15	333 ± 16	295 ± 29	204 ± 26	142 ± 15	94 ± 14	66 ± 13	83 ± 35	208 ± 129	324 ± 219	GT	GT
hIgG 8.8 vc_0101	3	212 ± 22	384 ± 35	567 ± 95	963 ± 204	GT	GT	GT	GT	GT	GT	GT	GT
	10	211 ± 20	354 ± 37	349 ± 48	198 ± 38	151 ± 31	165 ± 33	413 ± 128	922 ± 287	GT	GT	GT	GT

GT= group terminated due to large tumor size.

Example 17
Cysteine Mutant Generation for Site-Specific Conjugation

Site specific conjugation of linker-payloads to antibodies was done in order to improve homogeneous drug loading and avoid ADC subpopulations with altered antigen-binding or altered pharmacokinetics, often observed by conventional conjugation methods. One such site-specific conjugation method is to introduce cysteine residues at specific sites in the amino acid sequence of the target antibody. A number of amino acid positions in the constant heavy chain and constant light chain have been previously identified (see patent application USSN 61/580,169) and were substituted with a cysteine residue at the specific amino acid position in the hu08v1.0/1.0 antibody. All cysteine mutations were constructed by site-directed mutagenesis or overlapping PCR based on pSMED-hu08v1.0 and pSEMN3-hu08v1.0. Below is the list of cysteine mutants derived from hu08v1.0/1.0 and the respective SEQ ID numbers (Table 21).

Table 21

Antibody		Mutation Site	aa SEQ ID NO:
Single mutant	HC	L443C	28
		Q347C	29
	LC	kA111C	30
		kK183C	31
		kK188C	32
Double mutant	HC	L443C/K392C	33
		L443C/V422C	34
	HC/LC	L443C/kA111C	28/30
		L443C/kK183C	28/31
		Q347C/kA111C	29/30
		Q347C/kK183C	29/31

Example 18

Characterization of Cysteine Mutants

Cysteine mutants of hu08v1.0/1.0 were expressed either in transiently transfected HEK293 suspension cell cultures in freestyle 293 expression medium (Invitrogen, calsdad, CA) or in CHO cell culture stable pools. The antibodies were isolated from the cell culture medium by Protein A (ProA) chromatography under standard conditions. The column fractions were pooled and concentrated using a Millipore spin tube equipped with a 30,000 MWCO membrane. The protein was then loaded onto a size exclusion column (Superdex 200) equilibrated with PBS-CMF, pH 7.2. Peak fractions were pooled and concentrated using a Millipore spin tube equipped with a 30,000 MWCO membrane and finally filtered through a 0.22um filter. Data is shown in Table 22 from transient expression and in Table 23 from CHO cell stable pools. Wild type hu08v1.0/1.0 and its cysteine derivatives have comparable final yield

and purity after ProA capture, reach up to 99% purity after SEC and are stable after 2-3 cycles of freezing and thawing. This data demonstrates that the cysteine mutants maintain their expression profile and purification properties compared to hu08v1.0/1.0.

Table 22

Antibody Name	Final Yield (mg/L)	Purity (ProA capture)	Purity (SEC)	F/T 2-3 cycles
hu08v1.0/1.0	26.5	97%	ND	stable
hu08v1.0/1.0-L443C	56.0	94%	99%	stable
hu08v1.0/1.0-Q347C	30.0	97%	99%	stable
hu08v1.0/1.0-A111C	52.8	96.4%	99%	stable
hu08v1.0/1.0-K183C	44.4	97.0%	99%	stable
hu08v1.0/1.0-K188C	27.9	96.2%	99%	stable
hu08v1.0/1.0-K392C/L443C	28.0	85.0%	99%	stable
hu08v1.0/1.0-V422C/L443C	40.5	99.7%	ND	stable
hu08v1.0/1.0-Q347C/A111C	31.4	96.6%	99%	stable
hu08v1.0/1.0-L443C/A111C	43.7	ND	99%	stable
hu08v1.0/1.0-Q347C/K183C	29.0	97.2%	99%	stable
hu08v1.0/1.0-L443C/K183C	57.0	ND	99%	stable

Table 23

Antibody Name	Final Yield (mg/L)	Purity (ProA capture)	Purity (SEC)	F/T 2-3 cycles
hu08v1.0/1.0-Q347C	26.74	94.0%	99.0%	stable
hu08v1.0/1.0-K392C/L443C	38.33	89.0%	99.0%	stable
hu08v1.0/1.0	91.0	95%	99.0%	stable

Binding properties of hu08v1.0/1.0 cysteine mutants.

The binding properties of the cysteine mutants to hIL-13-Rα2 were evaluated by a standard ELISA and a competition ELISA. The results show that all of the Cys mutants have a similar ED50 compared to wild type hu08v1.0/1.0 (Table 23). This conclusion was confirmed by a competition ELISA with biotinylated ch08. Table 24 demonstrates that all the Cys mutants have a similar IC50 as wild type hu08, indicating that the binding affinity is the same as wild type hu08. ch07 was used as a negative control.

Table 24

	ED50 (nM)	IC50 (nM)
hu08	0.11	2.01
hu08/L443C	0.10	2.25
hu08/L443C/K392C	0.09	2.10
hu08/L443C/V422C	0.09	2.17
hu08/L443C/kA111C	0.12	2.32
hu08/L443C/kK183C	0.11	2.45
hu08/Q347C	0.10	2.07
hu08/Q347C/kA111C	0.10	2.44
hu08/Q347C/kK183C	0.13	2.41
hu08/kA111C	0.22	4.07
hu08/kK183C	0.11	2.47
hu08/kK188C	0.18	2.73
hu08/kD185A	0.108	2.364
ch07	0.10	2.11

Thermal stability of hu08v1.0/1.0 cysteine mutants.

The thermal stability of the anti-IL-13-Rα2 cysteine mutant mAbs was analyzed by Capillary Differential Scanning Calorimetry (DSC) using a MicroCal's Capillary-DSC system equipped with an autosampler (Northampton, MA). A standard protocol was utilized. The heat capacity difference between the sample cell and reference cell was recorded and analyzed using the non-2 states model fit to 3 thermal transitions in the Origin7.0 software (OriginLab, Northampton, MA). A baseline thermogram was also generated with PBS buffer in both the sample and reference cells, and used for subtraction of any system heat not associated with protein denaturation. The results in

Table 25 show that 2 single and 3 double cysteine mutant antibodies have comparable Tms as the parental hu08v1.0/1.0.

Table 25

hu08v1.0/1.0 and its cysteine mutants		DSC (differential scanning calorimetry)		
		Tm1 (^{0}C) CH2	**Tm2 (^{0}C) Fab**	**Tm3 (^{0}C) CH3**
Single Mutant	L443C	72.85±0.17	80.30±0.02	
	Q347C	72.78±0.15	80.18±0.02	
Double mutant	K392C/L443C	74.09±0.27	80.17±0.22	78.14±0.15
	Q347C/kK183C	72.59±0.23	79.82±0.12	
	L443C/kK183C	72.68±0.12	79.93±0.02	85.58±0.18
hu08v1.0/1.0		73.48±0.19	80.29±0.02	85.48±0.14

Thermal stability of hu08v1.0/1.0 cysteine mutant ADCs

Wild type hu08v1.0/1.0 and 5 cysteine mutants were conjugated with vc-0101 as described in US Provisional Patent Application 61/580,169. Thermal stability of all ADCs was measured by Capillary Differential Scanning Calorimetry (DSC, see details above). As shown below in Table 26, the Tm1 of the wild type hu08v1.0/1.0 vc-0101 conjugate are significantly lower (≥ 5^{0}C) than its naked antibody (see Table 8). The Tm2 of the ADC is about 1-2°C lower than naked antibody while the Tm3 is comparable. In terms of the hu08v1.0/1.0 cysteine mutants, Tm1, Tm2 and Tm3 of the vc-0101 conjugates are similar or slightly lower than (≤ 1-2^{0}C) its corresponding naked antibodies (see Table 25). This indicates that cysteine mutants are more thermal stable than wild type antibody after conjugation.

Table 26

Antibody		Conjugation		DSC (differential scanning calorimetry)			
		linker payload	DAR	Tm1 (^{0}C) CH2	Tm2 (^{0}C) Fab		Tm3 (^{0}C) CH3
Single mutant	L443C	vc-0101	2.1	73.27±0.19	79.29±0.01		
	Q347C	vc-0101	2.1	71.49±0.09	79.16±0.59		77.25±0.59
Double mutant	K392C/L443C	vc-0101	3.7	69.02±0.07	78.83±0.02		76.70±0.11
	Q347C/kK183C	vc-0101	4.3	70.15±0.08	78.21±0.12		76.20±1.10
	L443C/kK183C	vc-0101	4.0	71.30±0.13	77.85±0.02		84.16±0.11
hu08v1.0/1.0		vc-0101	3.2	68.42±0.09	77.77±0.10	79.45±0.02	85.03±0.07

Plasma and Glutathione stability of hu08v1.0/1.0 cysteine mutants conjugated with vc-0101.

Sample preparation for GSH stability: 30µg of a hu08-vc-0101 ADC or hu08-cys mutant-vc-0101 ADC in PBS was mixed with glutathione (GSH) solution to produce a final concentration of 0.5mM GSH. The ADC in 0.5mM GSH and control ADC (0mM GSH) were incubated at 37°C and sampled at 0, 3, and 6 days. TCEP (*tris*(2-carboxyethyl)phosphine) was used for reduction.

Sample preparation for mouse plasma stability: 90µg ADC sample in PBS was mixed with mouse plasma, diluted 1:1 with 20% MPER (Mammalian Protein Extraction Reagent). The ADC/plasma samples were incubated at 37°C and aliquots were taken at 0, 1, and 2 days and immune-precipitated with biotinylated recombinant hIL-13-Rα2 protein. The ADCs were eluted with 0.15% formic acid solution and neutralized with concentrated Tris HCl buffer to pH 7.8. Samples were deglycosylated by adding PNGaseF (Peptide: N-Glycosidase F) and reduced with TCEP.

LC/MS analysis procedure: Aliquots of the ADC/plasma and ADC/GSH stability samples were acidified by adding 0.1% formic acid solution with 10% acetonitrile and

followed by LC/MS analysis on an Agilent 1100 capillary HPLC coupled with a Water Xevo G2 Q-TOF mass spectrometer. The analytes were loaded onto a Zorbax Poroshell 300SB C8 column (0.5 mm X 75 mm, maintained at 80oC) with 0.1% formic acid, and eluted using a gradient of 20–40% buffer B (80% acetonitrile, 18% 1-propanol, 2% water with 0.1% formic acid) at a flow rate of 20µl/min over 5.5 minutes. Mass spectrometric detection was carried out in positive, sensitivity mode with capillary voltage set at 3.3 kV. Data analysis was performed with MaxEnt 1 function in MassLynx and intensities were used for loading calculation based on the following formula: Loading =2*[LC1/(LC0+LC1)]+2*HC1/(HC0+HC1+HC2)]+4*HC2/(HC0+HC1+HC2)].

The ADCs of hu08 and its cys mutant conjugated with vc-0101(from Table 26) were subjected to plasma and GSH stability assay. The results demonstrate that ADCs derived from cysteine mutants are more stable than the ADCs derived from the conventional conjugation technology (Table 27).

Table 27

Cys mutant-vc-0101 ADC		Plasma Stability % of loading Day 2	GSH Stability % of loading Day 6
Single mutant	L443C	95.0%	94.7%
	Q347C	95.0%	100.0%
Double mutant	K392C/L443C	89.7%	100.0%
	Q347C/kK183C	81.6%	97.4%
	L443C/kK183C	91.9%	86.8%
hu08		___	62.5%

Example 19

In Vitro Cytotoxicity Assay of Cys mutant ADCs

Cell lines expressing the IL-13-Rα2 antigen or the IL-13-Rα2 negative cell line, H460, were cultured with increasing concentrations of hu08v1.0/1.0 cysteine mutants conjugated with vc-0101 or mc-3377. After four days, viability of each culture was assessed. IC_{50} values were calculated by logistic non-linear regression and are presented in ng/mL (Tables 28a and 28b).

Table 28a

ADC (variant-vc-0101)	DAR	IC_{50} (ng/mL)	
		PC3MM2	A375
Wild type hu08	3.2	1.93±0.22	1.70±0.98
Q347C/kK183C	4.3	1.77±0.46	1.13±0.15
Q347C	2.1	2.66±0.60	1.65±0.09
L443C	2.1	2.40	1.41
K392C/L443C	3.7	1.41	0.63
L443C/kK183C	4.0	1.72	0.83

Table 28b

ADC (variant-mc-3377)	DAR	IC_{50} (ng Ab/mL)		
		PC3MM2	A375	H460
Wild type hu08	3.5	1.8	1.8	>400000
L443C	2.3	3.9	3.3	>400000
K392C+L443C	3.5	2.2	1.3	>400000
L443C+kK183C	4	1.9	2.3	>400000

Example 20

Subcutaneous Xenograft Models of Cys mutant ADCs

Female, athymic (nude) mice were injected s.c. with PC3MM2 tumor cells. Mice with staged tumors, approximately 0.2 to 0.5 g (n = 8 to 10 mice/treatment group) were administered a single dose at 1.5 or 4.5 mg/kg intravenously with normal saline (vehicle) cysteine mutants L443C-vc-0101, K392C/L443C-vc-0101, L443C/K183C-vc-0101, Q347C-vc-0101, or hAB-vc-0101, or 3, 6, and 12 mg/kg L443C-mc-3377, K392C/L443C-mc-3377, L443C/K183C-mc-3377. All ADCs were dosed based on Ab content. Tumors were measured at least once a week and their size ($mm^3 \pm$ SEM) was calculated as $mm^3 = 0.5 \times (\text{tumor width}^2) \times (\text{tumor length})$.

The data in Table 29 indicates that L443C-vc-0101, K392C/L443C-vc-0101, L443C/K183C-vc-0101, and hu08-vc-0101, at both 1.5 mg/kg and 4.5 mg/kg all inhibit the growth of PC3MM2 xenografts compared to vehicle control group. L443C/K183-vc-0101 is the most potent compound tested in the experiment as indicated with the longest monitoring time at Day 70 before the termination of the group at the dose level of 4.5 mg/kg.

Table 29

ADC	Dose Single dose	PC3MM2 xenograft, tumor volume (mm^3±SEM)											
		Day 0	Day 3	Day 6	Day 10	Day 13	Day 18	Day 21	Day 32	Day 42	Day 53	Day 63	Day 70
vehicle		341 ± 8	469 ± 25	681 ± 42	857 ± 60	1058 ± 115	1272 ± 167	GT	GT	GT	GT	GT	GT
L443C vc-0101	1.5	353 ± 26	421 ± 51	429 ± 65	428 ± 65	472 ± 93	567 ± 133	629 ± 176	GT	GT	GT	GT	GT
K392C+L443C vc-0101	1.5	343 ± 10	441 ± 31	417 ± 22	344 ± 23	356 ± 27	479 ± 57	565 ± 61	1219 ± 154	1411 ± 157	GT	GT	GT
L443C+Kk183C vc-0101	1.5	350 ± 18	431 ± 35	453 ± 49	396 ± 44	390 ± 49	464 ± 46	446 ± 54	842 ± 171	GT	GT	GT	GT
hu08-vc-0101	1.5	335 ± 16	451 ± 28	468 ± 47	439 ± 65	461 ± 86	600 ± 116	680 ± 135	1502 ± 406	GT	GT	GT	GT
L443C-vc-0101	4.5	358 ± 8	397 ± 13	334 ± 24	199 ± 25	164 ± 17	143 ± 23	124 ± 20	156 ± 47	242 ± 86	509 ± 193	618 ± 232	GT
K392C+L443C-vc-0101	4.5	351 ± 19	363 ± 27	353 ± 28	196 ± 10	169 ± 9	136 ± 17	118 ± 27	230 ± 98	318 ± 142	553 ± 235	GT	GT
L443C+Kk183C-vc-0101	4.5	343 ± 17	417 ± 32	397 ± 44	219 ± 18	156 ± 18	121 ± 8	93 ± 10	105 ± 27	175 ± 77	461 ± 221	423 ± 134	694 ± 222

GT= group terminated due to large tumor size.

The data in Table 30 indicates that Q347C-vc-0101 and Q347C/K183C-vc-0101, all inhibit the growth of PC3MM2 xenografts at both 1.5 mg/kg and 4.5 mg/kg compared to vehicle control group. Further, the data demonstrates that hu08MAC-0101 inhibits the growth of PC3MM2 xenografts at 1.5 mg/kg compared to the vehicle control group. The most potent compound was Q347C/K183C-vc-0101 as indicated at the dose level of 4.5 mg/kg with the longest monitoring time of Day 77. These results show that site-

specific vc-0101 conjugates are comparable or superior in efficacy to wild-type hu08-vc-0101 conjugates.

Table 30

ADC	Dose single dose	PC3MM2 xenograft, tumor volume (mm^3±SEM)									
		Day 0	Day 5	Day 8	Day 12	Day 15	Day 20	Day 30	Day 41	Day 55	Day 77
Vehicle	0	325 ± 9	590 ± 41	782 ± 79	1140 ± 142	GT	GT	GT	GT	GT	GT
Q347+kC183-vc-0101	1.5	337 ± 17	383 ± 39	324 ± 35	347 ± 46	381 ± 63	481 ± 89	770 ± 121	GT	GT	GT
Q347-vc-0101	1.5	333 ± 11	341 ± 14	308 ± 32	309 ± 39	352 ± 54	473 ± 79	757 ± 238	GT	GT	GT
hu08MAC-0101	1.5	328 ± 49	393 ± 96	352 ± 106	432 ± 124	556 ± 236	732 ± 305	GT	GT	GT	GT
Q347+kC183-vc-0101	4.5	336 ± 17	252 ± 19	171 ± 18	145 ± 13	128 ± 8	88 ± 14	113 ± 37	28 ± 28	75 ± 75	128 ± 128
Q347-vc-0101	4.5	338 ± 16	278 ± 28	176 ± 20	136 ± 16	130 ± 30	128 ± 41	267 ± 112	GT	GT	GT
hu08-vc-0101	1.5	333 ± 12	431 ± 40	281 ± 25	299 ± 32	362 ± 47	450 ± 58	956 ± 166	GT	GT	GT

GT= group terminated due to large tumor size

The data in Table 31 a and b indicates that L443C-mc-3377, K392C/L443C-mc-3377, L443C/K183C-mc-3377, and hu08-mc-3377 caused tumor regression in a dose-dependent manner compared to vehicle control group. L443C/K183C-mc-3377 was the most potent compound tested in the experiment as indicated with the small average tumor size at 12 mg/kg dose level compared to other compounds. In addition, there were 6 out of 8 animals without measurable tumors from the group treated with L443C/K183C-mc3377 at 12 mg/kg at Day 60.

Table 31a

ADC	Dose (mg/kg) single dose	PC3MM2 xenograft, tumor volume (mm^3 +/- SEM)								
		Day 0	Day 7	Day 14	Day 21	Day 28	Day 38	Day 45	Day 50	Day 60
vehicle	0	339 ± 19	1021 ± 112	1737 ± 194	GT	GT	GT	GT	GT	GT
C443 mc_3377	3	341 ± 24	350 ± 61	461 ± 82	601 ± 139	954 ± 250	GT	GT	GT	GT
	6	333 ± 31	352 ± 51	280 ± 54	366 ± 103	501 ± 147	1030 ± 307	GT	GT	GT
	12	338 ± 27	293 ± 45	177 ± 39	153 ± 22	278 ± 52	559 ± 117	752 ± 179	871 ± 267	GT
C392+C443 mc_3377	3	341 ± 26	387 ± 64	462 ± 132	496 ± 197	977 ± 381	GT	GT	GT	GT
	6	335 ± 31	237 ± 37	123 ± 37	133 ± 48	179 ± 85	469 ± 193	606 ± 252	768 ± 334	GT
	12	340 ± 23	312 ± 40	213 ± 45	150 ± 49	208 ± 79	261 ± 94	507 ± 242	695 ± 319	GT
C443+kC183 mc_3377	3	343 ± 27	386 ± 50	336 ± 60	452 ± 125	809 ± 245	GT	GT	GT	GT
	6	332 ± 28	311 ± 58	189 ± 32	135 ± 27	129 ± 37	237 ± 101	385 ± 185	491 ± 271	GT
	12	336 ± 32	238 ± 44	103 ± 30	49 ± 25	65 ± 26	61 ± 52	70 ± 55	109 ± 87	158 ± 123

Table 31 b

ADC	Dose (mg/kg) single dose	PC3MM2 xenograft, tumor volume (mm^3 +/- SEM)								
		Day 0	Day 7	Day 14	Day 21	Day 28	Day 38	Day 45	Day 50	Day 60
mc_3377	3	336 ± 29	340 ± 33	285 ± 75	436 ± 131	732 ± 265	GT	GT	GT	GT
	6	327 ± 35	231 ± 45	89 ± 29	30 ± 16	84 ± 25	184 ± 42	285 ± 108	402 ± 158	GT
	12	340 ± 28	180 ± 14	81 ± 28	52 ± 28	76 ± 45	171 ± 102	282 ± 179	354 ± 233	442 ± 328

GT= group terminated due to large tumor size

Example 21

Site-specific conjugation with MAC Technology

A method of preparing a multifunctional antibody conjugate (MAC) comprising an antibody or antigen binding fragment thereof has been described previously (WO2012/007896 and USSN 61/584,675.). An aspect of the present invention is a method of preparing a MAC utilizing an antibody or antigen binding fragment thereof that specifically binds to human IL-13Rα2 wherein the antibody has the mutation D185A at position 185 of the LC as shown in SEQ ID NO: 52, and the antibody is covalently conjugated to at least one drug moiety through a linker attached to a side chain of K188 of the LC of SEQ ID NO:49; said method comprising: covalently attaching the drug moiety using a PFP (Pentafluorophenyl) ester and reacting the Effector Moiety-linker-leaving group complex so formed with the antibody at a molar ratio of between about 3.5:1 to about 4.5:1 of drug moiety:antibody. In some aspects, the molar ratio is about 3.7:1 to about 4.3:1. The MAC described herein is designated as hu08MAC-0101 and

comprises the mutation D185A of the LC of SEQ ID NO: 52; and, the drug moiety 0101 (Example 13), which is conjugated to a side chain of the lysine residue at position 188 (K188) of the LC of SEQ ID NO: 52. In vitro activity of hu08MAC-0101 is shown in Table 17 (Example 15). In vivo activity in the PC3MM2 xenograft model is shown in Table 30 (Example 20).

WHAT IS CLAIMED:

1. An isolated antibody or antigen-binding fragment thereof that specifically binds to human IL-13-Rα2 wherein the antibody comprises: a heavy chain variable region comprising a CDR1, CDR2, and CDR3 of SEQ ID NO: 1 and, a light chain variable region comprising a CDR1, CDR2, and CDR3 of SEQ ID NO: 5.

2. An isolated antibody or antigen–binding fragment thereof that specifically binds to human IL-13-Rα2, wherein the antibody comprises:

(a) a heavy chain CDR1 comprising SEQ ID NO: 2;
(b) a heavy chain CDR2 comprising SEQ ID NO: 3;
(c) a heavy chain CDR3 comprising SEQ ID NO: 4;
(d) a light chain CDR1 comprising SEQ ID NO: 6 ;
(e) a light chain CDR2 comprising SEQ ID NO: 7; and,
(f) a light chain CDR3 comprising SEQ ID NO: 8.

3. The isolated antibody or antigen-binding fragment thereof of claim 2, wherein said isolated antibody further comprises the heavy chain variable region amino acid sequence of SEQ ID NO: 1.

4. The isolated antibody or antigen-binding fragment thereof of claim 2 or 3, wherein said isolated antibody further comprises the light chain variable region amino acid sequence of SEQ ID NO: 5.

5. The isolated antibody or antigen-binding fragment thereof of any one of claims 1-4, wherein said antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 50.

6. The isolated antibody or antigen-binding fragment thereof of any one of claims 1-5, wherein said antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 51.

7. An antibody-drug conjugate comprising a cytotoxic agent conjugated to the antibody or antigen-binding fragment thereof of any one of claims 1-6.

8. An antibody-drug conjugate of the formula:

Ab-(L-D)*p*

wherein:

(a) Ab is an isolated antibody or antigen-binding fragment thereof of any one of claims 1-6;

(b) L-D is a linker-drug moiety, wherein L is a linker, and D is a drug; and

(c) *p* is an integer from 1 to about 8.

9. The antibody-drug conjugate of claim 8 wherein L-D is selected from the group consisting of vc-0101 and mc-3377.

10. The antibody-drug conjugate of claims 8 or 9, wherein Ab comprises: (a) a heavy chain variable region comprising a CDR1, CDR2, and CDR3 of the VH sequence of SEQ ID NO: 1; and, (b) a light chain variable region comprising a CDR1, CDR2, and CDR3 of the VL sequence of SEQ ID NO: 5.

11. The antibody-drug conjugate of any one of claims 8 to 10, wherein Ab comprises: (a) a heavy chain CDR1 comprising SEQ ID NO: 2; (b) a heavy chain CDR2 comprising SEQ ID NO: 3; (c) a heavy chain CDR3 comprising SEQ ID NO: 4; (d) a light chain CDR1 comprising SEQ ID NO: 6; (e) a light chain CDR2 comprising SEQ ID NO: 7; and, (f) a light chain CDR3 comprising SEQ ID NO: 8.

12. An antibody-drug conjugate of the formula:

Ab-(L-D)*p*

wherein:

(a) Ab is an isolated antibody or antigen-binding fragment thereof of any one of claims 1-6;

(b) L-D is a linker-drug moiety, vc-0101; and

(c) *p* is an integer from 1 to about 4.

13. An antibody-drug conjugate of the formula:

Ab-(L-D)*p*

wherein:

(a) Ab is an isolated antibody or antigen-binding fragment thereof of any one of claims 1-6;

(b) L-D is a linker-drug moiety, mc-3377; and

(c) *p* is an integer from 1 to about 4.

14. A pharmaceutical composition comprising the antibody or antigen binding fragment thereof of any one of claims 1 to 6 and/or the antibody-drug conjugate of any one of claims 7-13 and a pharmaceutically acceptable carrier.

15. A method of treating an IL-13-Rα2 expressing cancer in a patient in need thereof, comprising administering to said patient the antibody-drug conjugate according to any one of claims 7-13.

16. The method of claim 15 wherein said cancer is selected from the group consisting of lung, colon, stomach, pancreatic, ovarian, malignant gliomas, and melanoma.

17. The antibody-drug conjugate of any one of claims 7-13 for use in therapy.

18. Use of an antibody-drug conjugate of any one of claims 7-13 in the manufacture of a medicament for therapy.

19. The use according to claims 17 or 18, wherein said use is for the treatment of an IL-13-Rα2 expressing cancer.

20. The use according to claim 19 wherein said cancer is selected from the group consisting of lung, colon, stomach, pancreatic, ovarian, malignant gliomas, and melanoma.

21. A nucleic acid that encodes the antibody or antigen-binding fragment thereof of any one of claims 1-6.

22. A vector comprising the nucleic acid of claim 21.

23. A host cell comprising the vector according to claim 22.

24. A process for producing an antibody comprising cultivating the host cell of claim 23 and recovering the antibody from a cell culture.

25. A process for producing an anti-IL-13-Rα2 antibody-drug conjugate of claim 9 comprising:

(a) linking a linker selected from the group consisting of maleimidocaproyl and maleimidocaproyl-Val-Cit-PABA to the drug;

(b) conjugating said linker-drug moiety to the antibody recovered from the cell culture of claim 24; and,

(c) purifying the antibody-drug conjugate.

26. An isolated antibody that competes with an antibody or antigen-binding fragment thereof of any one of claims 1-6 for specific binding to human IL-13-Rα2.

27. A method for predicting whether a subject with cancer will respond to an antibody-drug conjugate of any one of claims 7-13 comprising: determining whether a biological sample of said cancer from the subject expresses human IL-13-Rα2.

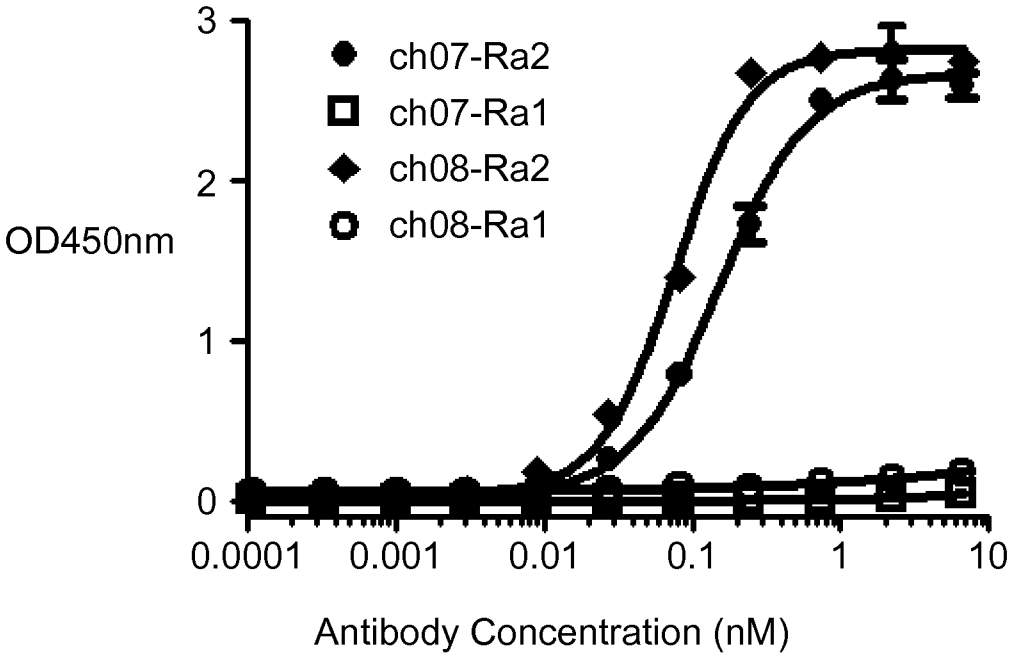
28. A process of determining the level of human IL-13-Rα2 in a biological sample comprising the steps of:

(a) testing a sample from a subject suspected to have cancer in a immunoassay using an antibody of any one of claims 1-6;

(b) determining the cell surface levels of human IL-13-Rα2 on said sample; and

(c) comparing the cell surface levels of human IL-13-Rα2 with that of a reference subject or standard.

FIG. 1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2
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ch07-Ra1
ch08-Ra2
ch08-Ra1
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0.001
0.01
0.1
1
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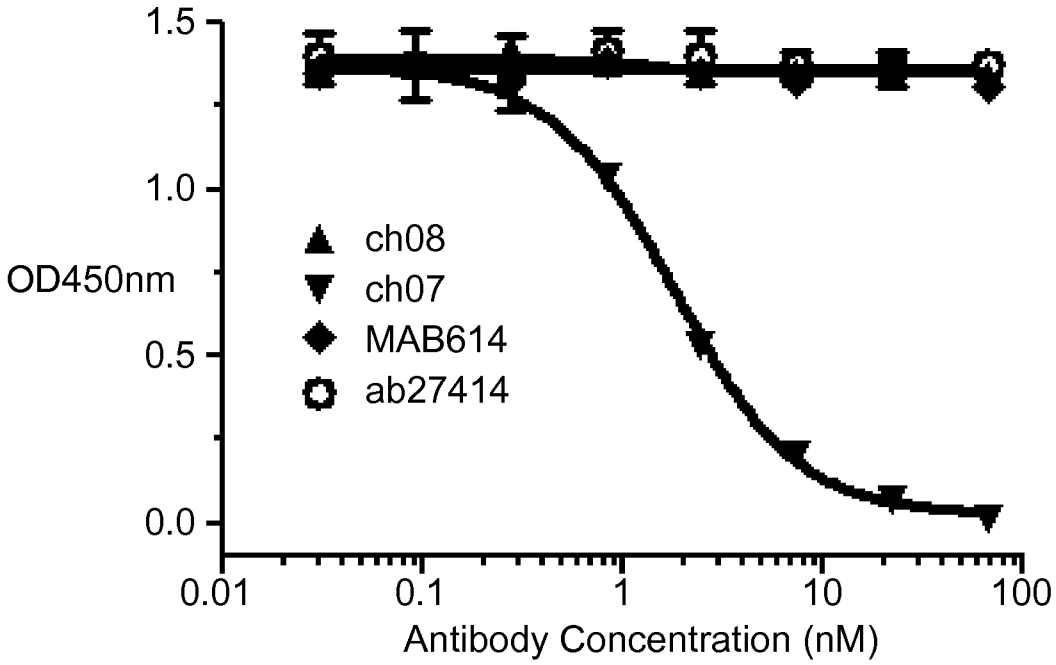
Antibody Concentration (nM)

FIG. 2A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MAB614
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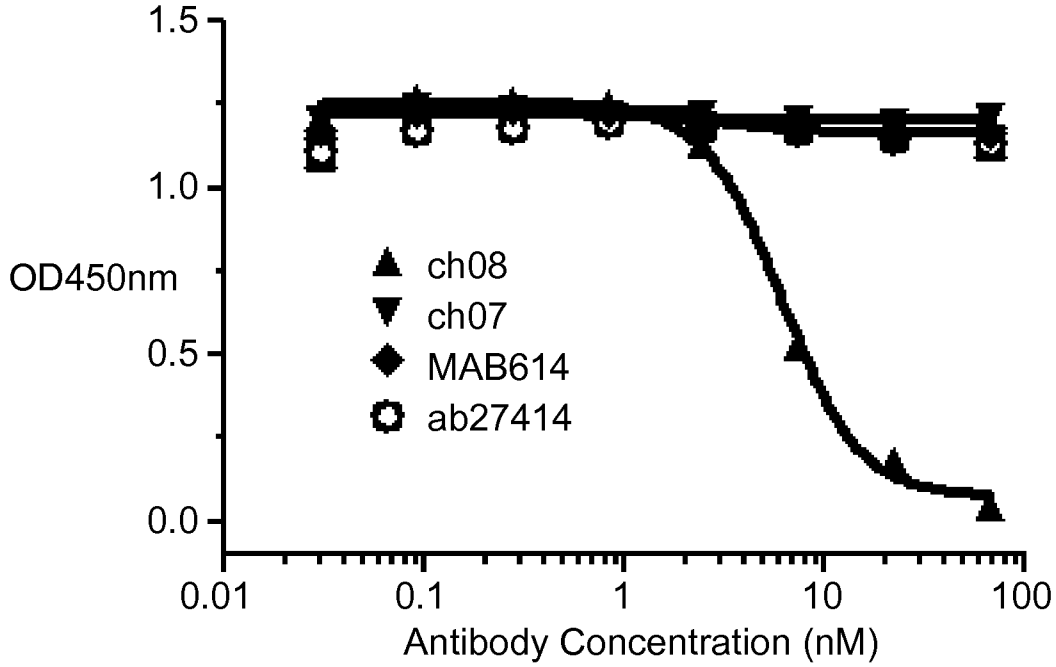
0.01
0.1
1
10
100
Antibody Concentration (nM)

FIG. 2B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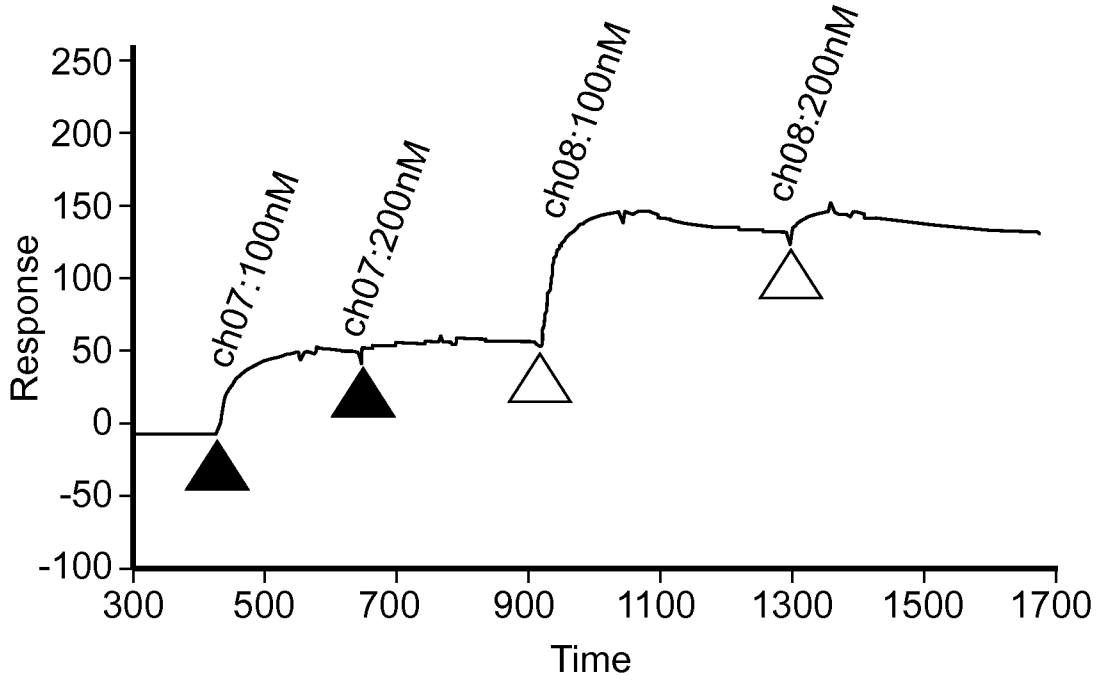
1.0
0.5
0.0
OD450nm
ch08
ch07
MAB614
ab27414
0.01
0.1
1
10
100
Antibody Concentration (nM)

FIG. 2C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200
150
100
50
0
-50
-100
Response
300
500
700
900
1100
1300
1500
1700
Time
ch07:100nM
ch07:200nM
ch08:100nM
ch08:200nM

FIG. 3

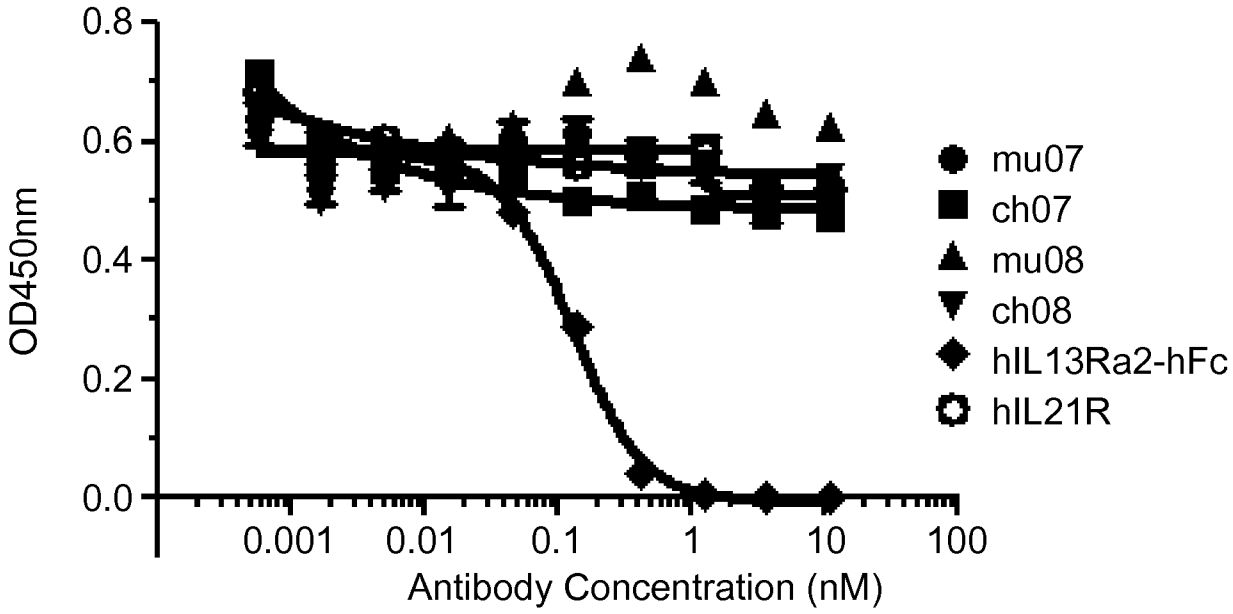
0.8
0.6
0.4
0.2
0.0
OD450nm
0.001
0.01
0.1
1
10
100
Antibody Concentration (nM)
mu07
ch07
mu08
ch08
hIL13Ra2-hFc
hIL21R

FIG. 4A

SEQ ID NO: 1 hu08-VH v1.0

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRNGMSWVRQAPGKGLEWVATVSSGGSYIYYADSVKGRFTISRDNAK
NSLYLQMNSLRAEDTAVYYCARQGTTALATRFFDVWGQGTLVTVSS

SEQ ID NO: 2 hu08 HCDR1

SRNGMS

SEQ ID NO: 3 hu08 HCDR2

TVSSGGSYIYYADSVKG

SEQ ID NO: 4 hu08 HCDR3

QGTTALATRFFDV

SEQ ID NO: 5 hu08-VL v1.0

DIQMTQSPSSLSASVGDRVTITCKASQDVGTAVAWYQQKPGKAPKLLIYSASYRSTGVPSRFSGSGSGTDFTLTISSL
QPEDFATYYCQHHYSAPWTFGGGTKVEIK

SEQ ID NO: 6 hu08 LCDR1

KASQDVGTAVA

SEQ ID NO: 7 hu08 LCDR2

SASYRST

SEQ ID NO: 8 hu08 LCDR3

QHHYSAPWT

SEQ ID NO: 9 hu07-VH v1.0

EVQLVESGGGLVQPGGSLRLSCAASGFTFTKYGVHWVRQAPGKGLEWVAVKWAGGSTDYNSALMSRFTISRDNAK
NSLYLQMNSLRAEDTAVYYCARDHRDAMDYWGQGTLVTVSS

SEQ ID NO: 10 hu07 HCDR1

TKYGVH

SEQ ID NO: 11 hu07 HCDR2

VKWAGGSTDYNSALMS

SEQ ID NO: 12 hu07 HCDR3

DHRDAMDY

SEQ ID NO: 13 hu07-VL v1.0

DIQMTQSPSSLSASVGDRVTITCTASLSVSSTYLHWYQQKPGKAPKLLIYSTSNLASGVPSRFSGSGSGTDFTLTISSL
QPEDFATYYCHQYHRSPLTFGGGTKVEIK

SEQ ID NO: 14 hu07 LCDR1

TASLSVSSTYLH

SEQ ID NO: 15 hu07 LCDR2

STSNLAS

SEQ ID NO: 16 hu07 LCDR3

HQYHRSPLT

FIG. 4B

SEQ ID NO: 17 hu08-VH v1.0

GAGGTGCAGCTGGTGGAGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCTCTCTGAGACTGTCTTGTGCCGCC
TCCGGCTTCACCTTCAGTAGGAATGGCATGTCTTGGGTGAGGCAGGCCCCTGGCAAGGGCCTGGAGTGGGTGG
CCACCGTTAGTAGTGGTGGTAGTTACATCTACTATGCAGACAGTGTGAAGGGGCGGTTCACCATCTCCAGGGAC
AACGCCAAGAACTCCCTGTACCTCCAGATGAACTCCCTGAGGGCCGAGGATACCGCCGTGTACTACTGTGCCA
GACAAGGGACTACGGCACTAGCTACGAGGTTCTTCGATGTCTGGGGCCAGGGCACCCTGGTGACCGTGTCCTC
T

SEQ ID NO: 18 hu08-VL v1.0

GACATCCAGATGACCCAGTCCCCCTCTTCTCTGTCTGCCTCTGTGGGCGACAGAGTGACCATCACCTGTAAGGC
CAGTCAGGATGTAGGTACTGCTGTAGCCTGGTATCAGCAGAAGCCTGGCAAGGCTCCCAAGCTGCTGATCTACT
CGGCATCCTACCGGTCCACTGGCGTGCCTTCCAGATTCTCCGGCTCTGGCTCTGGCACCGATTTCACCCTGACC
ATCTCCTCCCTCCAGCCTGAGGATTTCGCCACCTACTACTGCCAGCACCATTATAGTGCTCCGTGGACGTTTGG
CGGCGGAACAAAGGTGGAGATCAAG

SEQ ID NO: 19 hu08-VH v1.1

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRNGMSWVRQ**T**P**D**K**R**LEWVATVSSGGSYIYYADSVKGRFTISRDNAK
NSLYLQM**S**SLRAEDTAVYYCARQGTTALATRFFDVWGQGTLVTVSS

SEQ ID NO: 20 hu08-VH v1.1

GAGGTGCAGCTGGTGGAGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCTCTCTGAGACTGTCTTGTGCCGCC
TCCGGCTTCACCTTCAGTAGGAATGGCATGTCTTGGGTGAGGCAGACCCCTGACAAGCGCCTGGAGTGGGTGG
CCACCGTTAGTAGTGGTGGTAGTTACATCTACTATGCAGACAGTGTGAAGGGGCGGTTCACCATCTCCAGGGAC
AACGCCAAGAACTCCCTGTACCTCCAGATGAGCTCCCTGAGGGCCGAGGATACCGCCGTGTACTACTGTGCCA
GACAAGGGACTACGGCACTAGCTACGAGGTTCTTCGATGTCTGGGGCCAGGGCACCCTGGTGACCGTGTCCTC
T

SEQ ID NO: 21 hu08-VL v1.1

DIQMTQSPSSLSASVGDRVTITCKASQDVGTAVAWYQQ**I**PGKAPKLLIYSASYRSTGVP**D**RFSGSGSGTDF**S**FIISSLQ
PEDFATYYCQHHYSAPWTFGGGTKVEIK

SEQ ID NO: 22 hu08-VL v1.1

GACATCCAGATGACCCAGTCCCCCTCTTCTCTGTCTGCCTCTGTGGGCGACAGAGTGACCATCACCTGTAAGGC
CAGTCAGGATGTAGGTACTGCTGTAGCCTGGTATCAGCAGATCCCTGGCAAGGCTCCCAAGCTGCTGATCTACT
CGGCATCCTACCGGTCCACTGGCGTGCCTGACAGATTCTCCGGCTCTGGCTCTGGCACCGATTTCTCCTTTATC
ATCTCCTCCCTCCAGCCTGAGGATTTCGCCACCTACTACTGCCAGCACCATTATAGTGCTCCGTGGACGTTTGG
CGGCGGAACAAAGGTGGAGATCAAG

FIG. 4C

SEQ ID NO: 23 mu08 VH

EVQLVESGGDLVRPGGSLQLSCAASGFTFSRNGMSWVRQTPDRRLEWVATVSSGGSYIYYADSVKGRFTISRDNAR
NTLYLQMSSLKSEDTAMYYCARQGTTALATRFFDVWGAGTTVTVSS

SEQ ID NO: 24 mu08 VL

DIVMTQSHKFISTSVGDRVSITCKASQDVGTAVAWYQQIPGQSPKLLIYSASYRST
GIPDRFTGSGSGTDFSFIISSVQAEDLALYYCQHHYSAPWTFGGGTTLDIK

SEQ ID NO: 25 mu07 VH

QVQLKESGPGLVAPSQSLSINCTVSGFSLTKYGVHWIRQSPGKGLEWLGVKWAGGSTDYNSALMSRLTISKDNNKS
QVFLKMNSLQSDDSAMYYCARDHRDAMDYWGQGTSVTVSS

SEQ ID NO: 26 mu07 VL

QVVLTQSPAIMSASPGERVTMTCTASLSVSSTYLHWYHQKPGSSPKLWIYSTSNLASGVPARFSGSGSGTSYSLTISS
MEAEDAATYYCHQYHRSPLTFGSGTKLELK

SEQ ID NO: 27 cyno IL-13Ra2

DTEIKVNPPQDFEIVDPGYLGYLYLQWQPPLSLDNFKECTVEYELKYRNIGSETWTTIITKNLHYKDGFDLNKGIEAKIH
TLLPWQCTNGSEVQSSWAEATYWISPQGIPETKVQDMDCVYYNWQYLLCSWKPGIGVLLDTNYNLFYWYEGLDRAL
QCVDYIKVDGQNIGCRFPYLESSDYKDFYICVNGSSETKPIRSSYFTFQLQNIVKPLPPVCLTCTQESLYEIKLKWSIPL
GPIPARCFVYEIEIREDDTTLVTTTVENETYTLKITNETRQLCFVVRSKVNIYCSDDGIWSEWSDKQCWEVEELLKKTLL
LFLLPFGFILILVIFVTGLLL

SEQ ID NO: 28 hu08 HC 1.0 L443C

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRNGMSWVRQAPGKGLEWVATVSSGGSYIYYADSVKGRFTISRDNAK
NSLYLQMNSLRAEDTAVYYCARQGTTALATRFFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNY**K**TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS**C**SPGK

FIG. 4D

SEQ ID NO: 29 hu08 HC 1.0 Q347C

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRNGMSWVRQAPGKGLEWVATVSSGGSYIYYADSVKGRFTISRDNAK
NSLYLQMNSLRAEDTAVYYCARQGTTALATRFFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP**C**VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS**L**SPGK

SEQ ID NO: 30 hu08 KC 1.0 kA111C

DIQMTQSPSSLSASVGDRVTITCKASQDVGTAVAWYQQKPGKAPKLLIYSASYRSTGVPSRFSGSGSGTDFTLTISSL
QPEDFATYYCQHHYSAPWTFGGGTKVEIKRTVA**C**PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 31 hu08 KC 1.0 kK183C

DIQMTQSPSSLSASVGDRVTITCKASQDVGTAVAWYQQKPGKAPKLLIYSASYRSTGVPSRFSGSGSGTDFTLTISSL
QPEDFATYYCQHHYSAPWTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLS**C**ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 32 hu08 KC 1.0 kK188C

DIQMTQSPSSLSASVGDRVTITCKASQDVGTAVAWYQQKPGKAPKLLIYSASYRSTGVPSRFSGSGSGTDFTLTISSL
QPEDFATYYCQHHYSAPWTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLSKADYE**C**HKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 33 hu08 HC 1.0 L443C/K392C

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRNGMSWVRQAPGKGLEWVATVSSGGSYIYYADSVKGRFTISRDNAK
NSLYLQMNSLRAEDTAVYYCARQGTTALATRFFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNY**C**TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS**C**SPGK

SEQ ID NO: 34 hu08 HC 1.0 L443C/V422C

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRNGMSWVRQAPGKGLEWVATVSSGGSYIYYADSVKGRFTISRDNAK
NSLYLQMNSLRAEDTAVYYCARQGTTALATRFFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNY**K**TTPPVLDSDGSFFLYSKLTVDKSRWQQGN**C**FSCSVMHEALHNHYTQKSLS**C**SPGK

FIG. 4E

SEQ ID NO: 35 hu07-HC-v1.5

EVQLVESGGGLVQPGGSLRLSCAASGF**SL**TKYGVHWVRQAPGKGLEWV**G**VKWAGGSTDYNSALMSRFTIS**K**DNAK
NSLYLQMNSLRAEDTAVYYCARDHRDAMDYWGQGTLVTVSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP
APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 36 hu07-LC-v1.7

DIQMTQSPSSLSASVGDRVTITCTASLSVSSTYLHWYQQKPG**SS**PKL**W**IYSTSNLASGVPSRFSGSGSGT**SY**TLTISS
LQPEDFATYYCHQYHRSPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL
QSGNSQESVTEQDSKDSTYSLSSTLTLSKAAYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 37 hu07 VH 1.1

EVQLVESGGGLVQPGGSLRLSCAASGF**SL**TKYGVHWVRQAPGKGLEWV**G**VKWAGGSTDYNSALMSR**L**TISRDNAK
SSLYLQMNSLRAEDTAVYYCARDHRDAMDYWGQGTLVTVSS

SEQ ID NO: 38 hu07 VH 1.2

EVQLVESGGGLVQPGGSLRLSCAASGFTFTKYGVHWVRQAPGKGLEWVAVKWAGGSTDYNSALMSRFTIS**K**DNAK
NSLYLQMNSLRAEDTAVYYCARDHRDAMDYWGQGTLVTVSS

SEQ ID NO: 39 hu07 VH 1.3

EVQLVESGGGLVQPGGSLRLSCAASGF**SL**TKYGVHWVRQAPGKGLEWVAVKWAGGSTDYNSALMSRFTIS**K**DNAK
NSLYLQMNSLRAEDTAVYYCARDHRDAMDYWGQGTLVTVSS

SEQ ID NO: 40 hu07 VH 1.4

EVQLVESGGGLVQPGGSLRLSCAASGF**SL**TKYGVHWVRQAPGKGLEWVAVKWAGGSTDYNSALMSRFTISRDNAK
NSLYLQMNSLRAEDTAVYYCARDHRDAMDYWGQGTLVTVSS

SEQ ID NO: 41 hu07 VH 1.5

EVQLVESGGGLVQPGGSLRLSCAASGF**SL**TKYGVHWVRQAPGKGLEWV**G**VKWAGGSTDYNSALMSRFTIS**K**DNAK
NSLYLQMNSLRAEDTAVYYCARDHRDAMDYWGQGTLVTVSS

FIG. 4F

SEQ ID NO: 42 hu07 VL 1.1

DIQMTQSPSSLSASVGDRVTITCTASLSVSSTYLHWYQQKPG**SS**PKLLIYSTSNLASGVPSRFSGSGSGT**S**FTLTISSL
QPEDFATYYCHQYHRSPLTFGGGTKVEIK

SEQ ID NO: 43 hu07 VL 1.2

DIQMTQSPSSLSASVGDRVTITCTASLSVSSTYLHWYQQKPGKAPKL**W**IYSTSNLASGVPSRFSGSGSGTDFTLTISS
LQPEDFATYYCHQYHRSPLTFGGGTKVEIK

SEQ ID NO: 44 hu07 VL 1.3

DIQMTQSPSSLSASVGDRVTITCTASLSVSSTYLHWYQQKPGKAPKLLIYSTSNLASGVPSRFSGSGSGTD**Y**TLTISSL
QPEDFATYYCHQYHRSPLTFGGGTKVEIK

SEQ ID NO: 45 hu07 VL 1.4

DIQMTQSPSSLSASVGDRVTITCTASLSVSSTYLHWYQQKPGKAPKL**W**IYSTSNLASGVPSRFSGSGSGTD**Y**TLTISS
LQPEDFATYYCHQYHRSPLTFGGGTKVEIK

SEQ ID NO: 46 hu07 VL 1.5

DIQMTQSPSSLSASVGDRVTITCTASLSVSSTYLHWYQQKPG**SS**PKL**W**IYSTSNLASGVPSRFSGSGSGT**S**FTLTISSL
QPEDFATYYCHQYHRSPLTFGGGTKVEIK

SEQ ID NO: 47 hu07 VL 1.6

DIQMTQSPSSLSASVGDRVTITCTASLSVSSTYLHWYQQKPG**SS**PKLLIYSTSNLASGVPSRFSGSGSGT**SY**TLTISSL
QPEDFATYYCHQYHRSPLTFGGGTKVEIK

SEQ ID NO: 48 hu07 VL 1.7

DIQMTQSPSSLSASVGDRVTITCTASLSVSSTYLHWYQQKPG**SS**PKL**W**IYSTSNLASGVPSRFSGSGSGT**SY**TLTISS
LQPEDFATYYCHQYHRSPLTFGGGTKVEIK

SEQ ID NO:49 hu08 heavy chain constant region

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC
SVMHEALHNHYTQKSLSLSPGK

FIG. 4G

SEQ ID NO: 50 hu08-HC v1.0

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRNGMSWVRQAPGKGLEWVATVSSGGSYIYYADSVKGRFTISRDNAK
NSLYLQMNSLRAEDTAVYYCARQGTTALATRFFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 51 hu08 LC v1.0

DIQMTQSPSSLSASVGDRVTITCKASQDVGTAVAWYQQKPGKAPKLLIYSASYRSTGVPSRFSGSGSGTDFTLTISSL
QPEDFATYYCQHHYSAPWTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLSKAAYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 52 hu08 LC constant region Km(3) D77A mutant

TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAAY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 53 hu08 LC constant region 45, 77, 83 genus

TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN**x**LQSGNSQESVTEQDSKDSTYSLSSTLTLSKA**x**Y
EKHK**x**YACEVTHQGLSSPVTKSFNRGEC

Wherein x at position 45 is A or V, x at position 77 is selected from the group consisting of A, G, I, V, L, R, S, T, Q, P, N, M, H and W, and x at position 83 is L or V.

SEQ ID NO: 54 hu08 LC constant region Km(3) D77 genus mutation

TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA**x**Y
EKHKVYACEVTHQGLSSPVTKSFNRGEC

Wherein x at position 77 is selected from the group consisting of A, G, I, V, L, R, S, T, Q, P, N, M, H and W.

SEQ ID NO: 55 hu08 LC constant region Km(3) Wild Type

TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAD
YEKHKVYACEVTHQGLSSPVTKSFNRGEC

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2013/059786

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K47/48 C07K16/28
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 2013/072813 A2 (PFIZER [US]) 23 May 2013 (2013-05-23)	1-28
L	page 7, line 14 - page 9, line 10 page 69, line 21 - page 70, line 17 page 88, line 9 - page 94, line 12 page 233, line 25 - page 238, line 1 tables 3,5-7 sequences 9-12 ----- -/--	1-28

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 February 2014	25/02/2014
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Bruma, Anja

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2013/059786

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)

   - [ ] on paper
   - [X] in electronic form

   b. (time)

   - [X] in the international application as filed
   - [ ] together with the international application in electronic form
   - [ ] subsequently to this Authority for the purpose of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2013/059786

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RONALD J BERNARDI ET AL: "Immunonanoshells for targeted photothermal ablation in medulloblastoma and glioma: an in vitro evaluation using human cell lines", JOURNAL OF NEURO-ONCOLOGY, KLUWER ACADEMIC PUBLISHERS, BO, vol. 86, no. 2, 6 September 2007 (2007-09-06), pages 165-172, XP019555486, ISSN: 1573-7373 the whole document	1-28
A	BERNARD J ET AL: "Expression of interleukin 13 receptor in glioma and renal cell carcinoma: IL13Ralpha2 as a decoy receptor for IL13", LABORATORY INVESTIGATION, NATURE PUBLISHING GROUP, THE UNITED STATES AND CANADIAN ACADEMY OF PALHOLOGY, INC, vol. 81, no. 9, 1 September 2001 (2001-09-01), pages 1223-1231, XP002300524, ISSN: 0023-6837, DOI: 10.1038/LABINVEST.3780336 the whole document	1-28
A	WO 02/17968 A2 (US GOV HEALTH & HUMAN SERV [US]; PURI RAJ K [US]) 7 March 2002 (2002-03-07) claims 1-33 paragraphs [0011] - [0012], [0022] - [0029], [0046], [0084] - [0086], [0089] - [0092]	1-28
A	WO 2004/087758 A2 (NEOPHARM INC [US]; GATELY STEPHEN T [US]; WANASKI STEPHEN P [US]) 14 October 2004 (2004-10-14) claims 1-45 paragraphs [0004] - [0007], [0026] - [0029], [0045] - [0049]	1-28

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2013/059786

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		WO 2004087758 A2	14-10-2004

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A61K 47/48(2006.01)
C07K 16/28(2006.01)

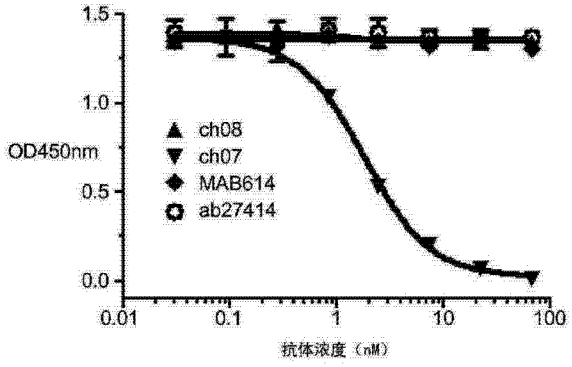
权利要求书2页 说明书41页
序列表34页 附图10页

(54)发明名称

抗 IL-13 受体 α2 抗体和抗体-药物缀合物

(57)摘要

本文公开了抗 IL-13-Rα2 抗体和抗体药物缀合物及制备和使用所述抗体和抗体药物缀合物的方法。



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1. 分离的抗体或其抗原结合片段，其特异性结合人 IL-13-Rα2，其中所述抗体包含：重链可变区以及轻链可变区，所述重链可变区包含 SEQ ID NO:1 的 CDR1、CDR2 和 CDR3，所述轻链可变区包含 SEQ ID NO:5 的 CDR1、CDR2 和 CDR3。

2. 分离的抗体或其抗原结合片段，其特异性结合人 IL-13-Rα2，其中所述抗体包含：

(a) 重链 CDR1，其包含 SEQ ID NO:2；

(b) 重链 CDR2，其包含 SEQ ID NO:3；

(c) 重链 CDR3，其包含 SEQ ID NO:4；

(d) 轻链 CDR1，其包含 SEQ ID NO:6；

(e) 轻链 CDR2，其包含 SEQ ID NO:7；以及

(f) 轻链 CDR3，其包含 SEQ ID NO:8。

3. 权利要求 2 的分离的抗体或其抗原结合片段，其中所述分离的抗体还包含 SEQ ID NO:1 的重链可变区氨基酸序列。

4. 权利要求 2 或 3 的分离的抗体或其抗原结合片段，其中所述分离的抗体还包含 SEQ ID NO:5 的轻链可变区氨基酸序列。

5. 权利要求 1-4 任一项的分离的抗体或其抗原结合片段，其中所述抗体包含重链，所述重链包含 SEQ ID NO:50 的氨基酸序列。

6. 权利要求 1-5 任一项的分离的抗体或其抗原结合片段，其中所述抗体包含轻链，所述轻链包含 SEQ ID NO:51 的氨基酸序列。

7. 抗体 - 药物缀合物，包含与权利要求 1-6 任一项的抗体或其抗原结合片段缀合的细胞毒性剂。

8. 具有分子式 Ab-(L-D)p 的抗体 - 药物缀合物，其中：

(a)Ab 是权利要求 1-6 任一项的分离的抗体或其抗原结合片段；

(b)L-D 是接头 - 药物部分，其中 L 是接头，D 是药物；以及

(c)p 是从 1 至约 8 的整数。

9. 权利要求 8 的抗体 - 药物缀合物，其中 L-D 选自 vc-0101 和 mc-3377。

10. 权利要求 8 或 9 的抗体 - 药物缀合物，其中 Ab 包含：(a) 重链可变区，所述重链可变区包含 SEQ ID NO:1 重链可变区序列的 CDR1、CDR2 和 CDR3；以及，(b) 轻链可变区，所述轻链可变区包含 SEQ ID NO:5 轻链可变区序列的 CDR1、CDR2 和 CDR3。

11. 权利要求 8-10 任一项的抗体 - 药物缀合物，其中 Ab 包含：(a) 重链 CDR1，其包含 SEQ ID NO:2；(b) 重链 CDR2，其包含 SEQ ID NO:3；(c) 重链 CDR3，其包含 SEQ ID NO:4；(d) 轻链 CDR1，其包含 SEQ ID NO:6；(d) 轻链 CDR2，其包含 SEQ ID NO:7；以及，(f) 轻链 CDR3，其包含 SEQ ID NO:8。

12. 具有分子式 Ab-(L-D)p 的抗体 - 药物缀合物，其中：

(a)Ab 是权利要求 1-6 任一项的分离的抗体或其抗原结合片段；

(b)L-D 是接头 - 药物部分，vc-0101；以及

(c)p 是从 1 至约 4 的整数。

13. 具有分子式 Ab-(L-D)p 的抗体 - 药物缀合物，其中：

(a)Ab 是权利要求 1-6 任一项的分离的抗体或其抗原结合片段；

(b)L-D 是接头 - 药物部分，mc-3377；以及

(c)p 是从 1 至约 4 的整数。

14. 药物组合物，包含权利要求 1-6 任一项的抗体或其抗原结合片段和 / 或权利要求 7-13 任一项的抗体 - 药物缀合物以及药物学可接受的载体。

15. 治疗有需要的患者中表达 IL-13-Rα2 的癌症的方法，包含向所述患者施用权利要求 7-13 任一项的抗体 - 药物缀合物。

16. 权利要求 15 的方法，其中所述癌症选自肺癌、结肠癌、胃癌、胰腺癌、卵巢癌、恶性胶质瘤和黑素瘤。

17. 权利要求 7-13 任一项的抗体 - 药物缀合物，用于治疗。

18. 权利要求 7-13 任一项的抗体 - 药物缀合物在制备治疗用药物中的用途。

19. 权利要求 17 或 18 的用途，其中所述用途是治疗表达 IL-13-Rα2 的癌症。

20. 权利要求 19 的用途，其中所述癌症选自肺癌、结肠癌、胃癌、胰腺癌、卵巢癌、恶性胶质瘤和黑素瘤。

21. 核酸，编码权利要求 1-6 任一项的抗体或其抗原结合片段。

22. 载体，包含权利要求 21 的核酸。

23. 宿主细胞，包含权利要求 22 的载体。

24. 生产抗体的方法，包含培养权利要求 23 的宿主细胞，并从细胞培养物中回收抗体。

25. 生产权利要求 9 的抗 IL-13-Rα2 抗体 - 药物缀合物的方法，包含：

(a) 将选自马来酰亚胺己酰基和马来酰亚胺己酰基 -Val-Cit-PABA 的接头与药物连接；

(b) 将所述接头 - 药物部分与权利要求 24 的从细胞培养物中回收的抗体缀合；以及

(c) 纯化抗体 - 药物缀合物。

26. 分离的抗体，其与权利要求 1-6 任一项的抗体或其抗原结合片段竞争特异性结合人 IL-13-Rα2。

27. 预测患癌症的受试者是否会对权利要求 7-13 任一项的抗体 - 药物缀合物应答的方法，包含：确定来自受试者的所述癌症的生物样品是否表达人 IL-13-Rα2。

28. 确定生物样品中的人 IL-13-Rα2 水平的方法，其包含以下步骤：

(a) 使用权利要求 1-6 任一项的抗体在免疫测定中测试来自疑似患有癌症的受试者的样品；

(b) 确定所述样品上的人 IL-13-Rα2 的细胞表面水平；以及

(c) 将人 IL-13-Rα2 的细胞表面水平与参考受试者或标准水平比较。

抗 IL-13 受体 α2 抗体和抗体 - 药物缀合物

技术领域

[0001] 本发明涉及抗 IL-13 受体 α2(IL-13-Rα2) 抗体和用于癌症治疗的抗体 - 药物缀合物。

[0002] 序列表

[0003] 本申请含有序列表，其通过 EFS 提交并通过引用整体并入本申请。

背景技术

[0004] 高水平 IL-13-Rα2 已在多种肿瘤细胞中被鉴定，包括胰腺、乳腺、卵巢和恶性胶质瘤。相比之下，只有一些类型的正常组织表达 IL-13-Rα2，并且仅在低水平表达。过去十年癌症治疗有所改善，手术、放疗和化疗是主要的治疗选择。这些治疗能够延长存活和 / 或缓解许多患者的症状，但对许多患者不可能治愈。仍存在对癌症其他的治疗选择的显著需要。

[0005] 因此，抗 IL-13-Rα2 抗体 - 药物缀合物符合在各种表达 IL-13-Rα2 的肿瘤细胞治疗中尚未满足的临床需求，其能显示出对表达 IL-13-Rα2 的肿瘤细胞的临床有效的细胞毒性效果，特别是不对不表达 IL-13-Rα2 的细胞显示不期望的效果。

[0006] 发明概述

[0007] 本发明提供抗 IL-13-Rα2 抗体 - 药物缀合物和用于癌症治疗的方法。

[0008] 本发明提供特异性结合人 IL-13-Rα2 的分离的抗体或抗原结合片段，其中抗体包含 ：包含 SEQ ID NO:1 重链可变区序列的 CDR1、CDR2 和 CDR3 的重链可变区，以及包含 SEQ ID NO:5 轻链可变区序列的 CDR1、CDR2 和 CDR3 的轻链可变区。

[0009] 本发明进一步提供特异性结合人 IL-13-Rα2 的分离的抗体或抗原结合片段，其中抗体包含 ：(a) 重链 CDR1，其包含 SEQ ID NO:2 ；(b) 重链 CDR2，其包含 SEQ ID NO:3 ；(c) 重链 CDR3，其包含 SEQ ID NO:4 ；(d) 轻链 CDR1，其包含 SEQ ID NO:6 ；(d) 轻链 CDR2，其包含 SEQ ID NO:7 ；以及，(f) 轻链 CDR3，其包含 SEQ ID NO:8。

[0010] 本发明进一步提供特异性结合人 IL-13-Rα2 的分离的抗体或抗原结合片段，其中所述分离的抗体还包含 SEQ ID NO:1 的重链可变区氨基酸序列和 SEQ ID NO:5 的轻链可变区氨基酸序列。

[0011] 本发明进一步提供特异性结合人 IL-13-Rα2 的分离的抗体或抗原结合片段，其中所述分离的抗体包含重链，所述重链含有 SEQ ID NO:50 的氨基酸序列，并且其中所述分离的抗体包含轻链，所述轻链含有 SEQ ID NO:51 氨基酸序列的。

[0012] 本发明进一步提供抗体 - 药物缀合物，其包含与特异性结合人 IL-13-Rα2 的抗体或其抗原结合片段缀合的细胞毒性剂。

[0013] 本发明进一步提供特异性结合人 IL-13-Rα2 的抗体 - 药物缀合物，其中所述缀合物具有分子式 ：Ab-(L-D)p，其中 (a)Ab 是本发明的抗体或其抗原结合片段 ；(b)L-D 是接头 - 药物部分，其中 L 是接头，D 是药物 ；以及 (c)p 是 1 至约 8 的整数。

[0014] 本发明进一步提供特异性结合人 IL-13-Rα2 的抗体 - 药物缀合物，其中接头选自

马来酰亚胺己酰基 (maleimidocaproyl)(mc) 和马来酰亚胺己酰基 -Val-Cit-PABA(vc)。

[0015] 本发明进一步提供特异性结合人 IL-13-Rα2 的抗体 - 药物缀合物，其中接头 - 药物部分具有如实施例 14 中所示的命名为 vc-0101 或 mc-3377 的分子式。

[0016] 本发明进一步提供特异性结合人 IL-13-Rα2 的抗体 - 药物缀合物，其中 Ab 包含：(a) 包含 SEQ ID NO:1 重链可变区序列的 CDR1、CDR2 和 CDR3 的重链可变区；以及，(b) 包含 SEQ ID NO:5 轻链可变区序列的 CDR1、CDR2 和 CDR3 的轻链可变区。

[0017] 本发明进一步提供特异性结合人 IL-13-Rα2 的抗体 - 药物缀合物，其中 Ab 包含：(a) 重链 CDR1，其包含 SEQ ID NO:2；(b) 重链 CDR2，其包含 SEQ ID NO:3；(c) 重链 CDR3，其包含 SEQ ID NO:4；(d) 轻链 CDR1，其包含 SEQ ID NO:6；(d) 轻链 CDR2，其包含 SEQ ID NO:7；以及，(f) 轻链 CDR3，其包含 SEQ ID NO:8。

[0018] 本发明进一步提供抗体 - 药物缀合物，其中 L-D 选自 vc-0101、mc-3377、mc-0131、MalPeg-6121、MalPeg-0131、mc-6121、vc-3906、vc-6780、mc-8261、mc3906 和 MalPeg-8261。

[0019] 本发明进一步提供特异性结合人 IL-13-Rα2 的抗体 - 药物缀合物，其中所述缀合物利用在改造的半胱氨酸残基上的位点特异性缀合并且具有分子式：Ab-(L-D)p，或其药物学可接受的盐，其中，(a)Ab 是抗体或其抗原结合片段，其包含重链可变区，所述重链可变区包含 SEQ ID NO:1 所示重链可变区序列的 CDR1、CDR2 和 CDR3；轻链可变区，所述轻链可变区包含 SEQ ID NO:5 所示轻链可变区序列的 CDR1、CDR2 和 CDR3；以及改造的 Fc 区，所述改造的 Fc 区包含选自 SEQ ID NO:33 和 SEQ ID NO:34 的氨基酸序列的至少一对氨基酸取代；或改造的 Fc 区以及至少一个改造的轻链恒定区，所述改造的轻链恒定区选自 L443C(SEQ ID NO:28)、Q347C(SEQ ID NO:29)、kK183C(SEQ ID NO:31)、L443C/kA111C(SEQ ID NO:28 和 30)、L443C/kK183C(SEQ ID NO:28 和 31)、Q347C/kA111C(SEQ ID NO:29 和 30) 以及 Q347C/kK183C(SEQ ID NO:29 和 31)；(b)L-D 是接头 - 药物部分，其中 L 是接头，D 是药物；以及 (c) p 是 1 至约 8 的整数。

[0020] 本发明进一步提供上述抗体 - 药物缀合物，其利用在改造的半胱氨酸残基上的位点特异性缀合，其中接头 - 药物部分具有如实施例 14 中所示的命名为 vc-0101 或 mc-3377 的分子式，或其药物学可接受的盐或溶剂化物形式，并且 p 是约 4 的整数。

[0021] 本发明进一步提供本发明的抗体 - 药物缀合物，其利用多功能抗体缀合物 (MAC) 技术，其中所述抗体或其抗原结合部分特异性结合人 IL-13Rα2，其中抗体在 SEQ ID NO:52 所示的轻链恒定区 (LC) 的 185 位具有突变 D185A，并且抗体通过接头共价缀合至至少一个药物部分，所述接头与 SEQ ID NO:49 的 LC 的 K188 的侧链连接；其中药物部分具有如实施例 13 中所示的命名为 0101 或 3377 的分子式，或其药物学可接受的盐或溶剂化物形式，并且 p 是整数，其范围的下限可选自约 1.5、约 1.6、约 1.7、约 1.8、约 1.9 和约 2.0，其范围的上限可选自约 2.0、约 2.1、约 2.2、约 2.3、约 2.4、约 2.5。在一些方面，p 是约 2。

[0022] 本发明进一步提供药物组合物，其包含本发明的抗体 - 药物缀合物和药物学可接受的载体。

[0023] 本发明进一步提供治疗有需要的患者中表达 IL-13-Rα2 的癌症的方法，包含向所述患者施用本发明的抗体 - 药物缀合物。

[0024] 本发明进一步提供治疗表达 IL-13-Rα2 的癌症的方法，其中所述癌症选自膀胱癌、乳腺癌、宫颈癌、结肠癌、恶性胶质瘤、子宫内膜癌、肾癌、肺癌、食管癌、卵巢癌、前列腺

癌、胰腺癌、黑素瘤、胃癌和睾丸癌。

[0025] 更具体地，本发明提供治疗表达 IL-13-Rα2 的癌症的方法，其中所述癌症选自肺癌、结肠癌、胃癌、胰腺癌、卵巢癌、恶性胶质瘤和黑素瘤。

[0026] 本发明进一步提供用于治疗的本发明的抗体 - 药物缀合物。

[0027] 本发明进一步提供本发明的抗体 - 药物缀合物在制备治疗用药物中的用途。

[0028] 本发明进一步提供本发明的抗体 - 药物缀合物的用途，其中所述用途是治疗表达 IL-13-Rα2 的癌症。

[0029] 本发明进一步提供编码 IL-13-Rα2 抗体的核酸，包含所述核酸的载体，以及包含所述载体的宿主细胞。

[0030] 本发明进一步提供生产 IL-13-Rα2 抗体的方法，其中所述方法包含培养包含上述载体的宿主细胞，并从细胞培养物中回收抗体。

[0031] 本发明进一步提供生产 IL-13-Rα2 抗体 - 药物缀合物的方法，包含 :(a) 将选自马来酰亚胺己酰基和马来酰亚胺己酰基 -Val-Cit-PABA 的接头与选自 0101 和 3377 的药物连接产生接头 - 药物部分 ;(b) 将所述接头 - 药物部分与上述从细胞培养物中回收的抗体缀合 ;以及，(c) 纯化抗体 - 药物缀合物。

[0032] 本发明进一步提供分离的抗体，其与本发明的抗体或其抗原结合片段竞争特异性结合人 IL-13-Rα2。

[0033] 本发明进一步提供抗体 - 药物缀合物，其包含特异性结合人 IL-13-Rα2 的本发明的抗体或其抗原结合片段。

[0034] 本发明进一步提供预测患癌症的受试者是否会对本发明的抗体 - 药物缀合物应答的方法，包含 :确定来自受试者的所述癌症的生物样品是否表达 hIL-13-Rα2。

[0035] 本发明进一步提供确定生物样品中的 hIL-13-Rα2 水平的方法，包含以下步骤 : 使用本发明的抗体在免疫测定中测试来自疑似患有癌症的受试者的样品 ;确定所述样品上的 hIL-13-Rα2 的细胞表面水平 ;以及，将 hIL-13-Rα2 的细胞表面水平与参考受试者或标准水平比较。

[0036] 本发明进一步提供治疗表达 IL-13-Rα2 的癌症的方法，所述方法包含 :确定生物样品中的 hIL-13-Rα2 水平，其包含以下步骤 :使用本发明的抗体在免疫测定中测试来自疑似患有癌症的受试者的样品 ;确定所述样品上的 hIL-13-Rα2 的细胞表面水平 ;将 hIL-13-Rα2 的细胞表面水平与正常参考受试者或标准水平比较 ;以及施用本发明的抗体 - 药物缀合物。

附图说明

[0037] 图 1 :嵌合抗体 ch07 和 ch08 与 hIL-13-Rα2 而非 hIL-13Rα1 的结合特异性。

[0038] 图 2A 和图 2B :ch07、ch08 和抗体 MAB614 及 ab27414 具有不同的 IL-13Rα2 结合表位。

[0039] 图 2C :Biacore 分析表明 ch07 和 ch08 缺乏结合竞争。

[0040] 图 3 :ch07 和 ch08 是非中和抗体。

[0041] 图 4A - 4G :SEQ ID NO:1 - 55。

[0042] 发明详述

[0043] 本发明提供抗用于癌症治疗的 IL-13-Rα2 抗体 - 药物缀合物。为了更容易理解本发明，首先定义某些术语和通用技术。

[0044] 本发明中所用的所有氨基酸缩写均为美国专利商标局所接受的，如 37C.F.R. §1.822(d)(1) 所规定。

[0045] 除非本文另有限定，所用的与本发明相关的科技术语应当具有本领域技术人员通常理解的含义。另外，除非文中另有要求，单数术语应包括复数，复数术语应包括单数。通常，本文所述的与细胞和组织培养、分子生物学、免疫学、微生物学、遗传学以及蛋白和核酸化学和杂交相关的所用术语及其技术是本领域熟知和通常使用的。

[0046] 除非另有说明，本发明的方法和技术通常按照本领域熟知的常规方法进行，并如遍及本发明所引用和讨论的各种概括和更具体的参考文献中所述。参见例如 Sambrook J.& Russell D.Molecular Cloning:A Laboratory Manual,3rd ed.,Cold Spring Harbor Laboratory Press,Cold Spring Harbor,N.Y.(2000) ;Ausubel 等人，Short Protocols in Molecular Biology:A Compendium of Methods from Current Protocols in Molecular Biology,Wiley,John & Sons,Inc.(2002) ;Harlow 和 Lane Using Antibodies:A Laboratory Manual,Cold Spring Harbor Laboratory Press,Cold Spring Harbor,N.Y.(1998) ；以及 Coligan 等人，Short Protocols in Protein Science,Wiley,John & Sons,Inc.(2003)。

[0047] “抗体”或“Ab”是能通过至少一个抗原识别位点特异性结合标靶标，诸如碳水化合物、多核苷酸、脂质、多肽等的免疫球蛋白分子，该抗原识别位点位于所述免疫球蛋白分子的可变区。文中所使用的术语“抗体”不仅包含完整的多克隆或单克隆抗体，还包含其任意抗原结合片段（即“抗原结合部分”）或单链、包含抗体的融合蛋白质及任何其他含有抗原识别位点的免疫球蛋白分子的修饰构型，例如但不限于，scFv、单结构域抗体（例如鲨鱼和骆驼抗体）、maxibody、minibody、胞内抗体、双特异抗体、三特异抗体、四特异抗体、v-NAR 和 bis-scFv（参见例如 Hollinger and Hudson,2005,Nature Biotechnology 23(9):1126-1136)。抗体包括任何类型的抗体，诸如 IgG、IgA 或 IgM（或其亚型），且所述抗体不需要具有任何特定类型。根据抗体的重链恒定区的氨基酸序列，免疫球蛋白可被分成不同类型。有五种主要的免疫球蛋白类型 :IgA、IgD、IgE、IgG 和 IgM，其中几个类型可进一步被分成亚型（同种型），例如 IgG1、IgG2、IgG3、IgG4、IgA1 和 IgA2。对应不同类型的免疫球蛋白的重链恒定区分别被称为 α、δ、ε、γ 和 μ。不同类型的免疫球蛋白的亚单元结构及三维构型是周知的。

[0048] 术语“分离的”指基本上脱离其天然环境的分子。例如，分离的抗体是基本上不含细胞材料或其他来自该细胞的蛋白或其所来源的组织来源。

[0049] 术语抗体的“抗原结合片段”用于本文指完整抗体的一或多种片段，其保留与给定抗原（例如靶 IL-13Rα2）特异性结合的能力。抗体的抗原结合功能能通过完整抗体的片段执行。术语抗体的“抗原结合部分”中包含的结合片段的实例包括 Fab、Fab'、$F(ab')_2$ 和由 VH 及 CH1 结构域组成的 Fd 片段、由抗体单臂的 VL 和 VH 结构域组成的 Fv 片段、单结构域抗体 (dAb) 片段 (Ward 等人，1989 Nature 341:544-546)，以及分离的互补决定区 (CDR)。

[0050] 抗体的“可变区”是指单独或组合的抗体轻链 (VL) 的可变区或抗体重链 (VH) 的可变区。如本领域所知，重链及轻链的可变区各由四个框架区 (FR) 组成，该四个框架

区由三个互补决定区 (CDR1、CDR2 和 CDR3) 又名高变区所连接。如果期望受试者可变区变体，尤其是在 CDR 区外（即框架区内）具有氨基酸残基的取代，可通过将受试者可变区与其他抗体（其在与受试者可变区相同的规范类中含有 CDR1 和 CDR2 序列）的可变区比较，鉴定适合的氨基酸取代，优选保守氨基酸取代 (Chothia and Lesk, J Mol Biol 196(4):901-917, 1987)。当选择受试者 CDR 侧翼的 FR 时，例如当人源化或优化抗体时，优选来自在相同的规范类中含有 CDR1 和 CDR2 序列的抗体的 FR。

[0051] 可变区的“CDR "是位于可变区内的氨基酸残基，其根据卡巴 (Kabat) 定义、柯西亚 (Chothia) 定义、卡巴及柯西亚的累积定义、AbM 定义、接触定义和 / 或构形定义，或本领域众所周知的任何 CDR 确定方法加以识别。抗体 CDR 可能被鉴定为最先由卡巴等人所定义的超变区。参见例如 Kabat 等人，1992, Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, NIH, Washington D.C.。CDR 的位置也可能被鉴定为最先由柯西亚等人所描述的结构性环结构。参见例如 Chothia 等人，Nature342:877-883, 1989。其它鉴定 CDR 的方法包括“AbM 定义”，该方法为卡巴法及柯西亚法的折衷，是源自利用 Oxford Molecular 的 AbM 抗体模型软件（现为 Accelrys®），或如 MacCallum 等人，J. Mol. Biol. 262:732-745, 1996 所述的基于抗原接触观察的 CDR 的“接触定义”。在此处称为 CDR 的“构形定义”的另一方法中，CDR 的位置可能被鉴定为对抗原结合造成焓贡献的残基。见例如 Makabe 等人，Journal of Biological Chemistry, 283:1156-1166, 2008。其它 CDR 边界定义仍可能不严格遵守上述方法中之一，但将与至少部分的卡巴 CDR 重叠，不过它们可能根据特定残基或残基群或甚至整个 CDR 不显著影响抗原结合的预测或实验结果被缩短或延长。此处所使用的 CDR 可能指由本领域已知的任何方法（包括多种方法的组合）所定义的 CDR。此处所使用的方法可利用根据任意这些方法所定义的 CDR。以包含超过一种 CDR 的任何给定实施方式而言，CDR 可根据任意的卡巴、柯西亚、延长、AbM、接触和 / 或构形定义加以定义。

[0052] 术语“IgG Fc 区”、“Fc 区”、“Fc 结构域”和“Fc”在本文可互换使用，指 IgG 分子的部分，其对应于通过 IgG 分子的木瓜蛋白酶消化获得的可结晶的片段。Fc 区由通过二硫键连接的 IgG 分子的两个重链的 C 末端一半组成。其不具有抗原结合活性，但含有碳水化合物部分以及补体和 Fc 受体（包括 FcRn 受体）结合位点（见如下）。Fc 片段含有完整的第二恒定结构域 CH2（人 IgG1 的残基 231-340，根据 Kabat 计数系统）和第二恒定结构域 CH3（残基 341-447）。

[0053] 术语“工程化 Fc 多肽”、“工程化 Fc 区”和“工程化 Fc”和“Fc”在本文可互换使用，指 Fc 多肽或其部分，其包含至少一个突变，例如氨基酸取代，引入缀合位点。优选地，所述突变引入半胱氨酸，取代该位置处天然存在的氨基酸残基，突变在此产生用于一个部分与 Fc 缀合的反应位点（例如反应性巯基）。

[0054] 术语“单克隆抗体”或“mAb”是指来自单拷贝或克隆，包括例如任意真核、原核或噬菌体克隆的抗体，而并非藉此制备其的方法。优选地，本发明的单克隆抗体存在于同源或实质性同源的群体中。

[0055] “人源化”抗体是指非人（例如鼠）抗体形式，其为嵌合免疫球蛋白、免疫球蛋白链或其片段（诸如 Fv、Fab、Fab'、$F(ab')_2$ 或抗体的其他抗原结合子序列），其含有来源于非人免疫球蛋白的最小序列。优选地，人源化抗体是人免疫球蛋白（接受者抗体），其中源

自接受者的互补决定区 (CDR) 的残基被源自非人物种（捐赠者抗体）诸如具有期望的特异性、亲和性及能力的小鼠、大鼠或兔的 CDR 的残基所取代。

[0056] “人抗体”或“完全人抗体”是指来自携带人抗体基因的转基因小鼠或来自人细胞的那些抗体。

[0057] 术语“嵌合抗体”意在指其中的可变区序列来自一个物种并且恒定区序列来自另一个物种的抗体，例如其中可变区序列来自小鼠抗体并且恒定区序列来自人抗体的抗体。

[0058] “治疗剂”是对癌细胞或活化免疫细胞显示细胞毒性、细胞生长抑制和 / 或免疫调制效果的试剂。治疗剂的实例包括细胞毒性剂、化疗剂、细胞生长抑制剂和免疫调制剂。

[0059] “化疗剂”是用于癌症治疗的化合物。

[0060] “细胞毒性效果”指排除、消灭和 / 或杀死靶细胞。“细胞毒性剂”指对细胞具有细胞毒性和 / 或细胞生长抑制效果的试剂。

[0061] “细胞生长抑制效果”指抑制细胞增殖。“细胞生长抑制剂”指对细胞具有细胞生长抑制效果，由此抑制特定细胞亚群的生长和 / 或扩展的试剂。

[0062] “抗体 - 药物缀合物”或“ADC”指抗体或其抗体片段，包括结合 IL-13Rα 2 并与细胞毒性、细胞生长抑制和 / 或治疗剂缀合的抗体衍生物。

[0063] “抗 IL-13Rα 2 抗体 - 药物缀合物”指抗 IL-13Rα 2 抗体或其抗原结合片段，如本文所述与细胞毒性药物 (D) 通过接头 (L) 连接。

[0064] “接头 (L)”描述了抗体与药物的直接或间接连接。接头与 mAb 的结合可通过多种方式实现，例如通过表面赖氨酸，与氧化碳水化合物还原性偶联，以及通过由还原链间二硫键释放的半胱氨酸残基。各种 ADC 连接系统是本领域已知的，包括腙 -、二硫 - 和基于肽的连接。

[0065] “药物 (D)”是具有生物学或可检测活性的任意物质，例如治疗剂、可检测标记、结合剂等，以及药物前体 (prodrug)，其在体内代谢成活性剂。术语药物、药物部分、有效负载 (payload) 和化合物可互换使用。

[0066] “L-D”是由细胞毒性药物 (D) 与接头 (L) 连接产生的接头 - 药物部分。

[0067] 术语“表位”是指能够被抗体在抗体的一或多个抗原结合区域识别和结合的分子部分。表位通常由分子的化学活性表面基团例如氨基酸或糖侧链组成并且具有特定的三维结构特征和特定的电荷特征。本文所用术语“抗原性表位”定义为抗体可特异性结合（如可通过本领域内熟知的任何方法例如通过常规免疫测定法确定的）的多肽部分。“非线性表位”或“空间表位”包含被特异于该表位的抗体结合的抗原性蛋白中的非连续多肽（或氨基酸）。一旦抗原上的期望的表位确定，可以产生针对该表位的抗体，例如使用本说明书中描述的技术。在发现过程中，抗体的产生和表征可阐明关于期望的表位的信息。由该信息，继而可以竞争性筛选结合相同表位的抗体。实现这一点的方式是进行竞争和交叉竞争研究以发现与其他抗体竞争或交叉竞争的抗体，例如竞争结合抗原的抗体。

[0068] 本文所用术语“结合亲和力 (K_D)”意在指特定抗原 - 抗体相互作用的解离速率。K_D 是解离速率 (K_d)，又名“解离速率 (K_{off})”，与结合速率 (K_a)，或“结合速率 (K_{on})”的比率。因此，K_D 等于 K_{off}/K_{on} 并表示为摩尔浓度 (M)。K_D 越小，结合亲和力越强。因此，1μM 的 K_D 表明与 1nM 的 K_D 相比为弱结合亲和力。抗体的 K_D 值可使用本领域熟知的方法确定。确定抗体 K_D 的一种方法是使用表面等离子体共振，通常使用生物传感器系统例如 Biacore® 系统。

[0069] 本文所用术语“特异性结合”是指抗体与 IL-13-Rα2 抗原之间的结合，并且抗体以小于约 30nM 的 K_D（由表面等离子体共振 (SPR) 在 25℃确定）结合 IL-13-Rα2 抗原。

[0070] 本文所用“药物学可接受的盐”是指分子或大分子的药物学可接受的有机或无机盐。

[0071] 术语“效能”是生物学活性的量度并可命名为 IC_{50}，或需要如实施例 15 所述抑制 IL-13-Rα2 阳性细胞系 50％的生长的针对抗原 IL-13-Rα2 的抗体或抗体药物缀合物的抑制性浓度。

[0072] “EC_{50}”是结合能力的量度并定义为需要在基线和最大值之间产生半数响应的抗体或抗体 - 药物缀合物的半数最大有效浓度。

[0073] 本文所用短语“有效量”或“治疗有效量”是指实现期望的治疗结果必需（施用的剂量、时间段和方式）的量。有效量至少是对受试者产生治疗益处必需的活性剂的最小量，但低于毒性量。

[0074] 本文所用关于本发明抗体的生物活性的术语“抑制”或“中和”是指抗体实质性对抗、阻止、防止、抑制、减缓、破坏、消除、停止、减少或逆转例如被其抑制的，包括但不限于生物学活性的进展或严重性的能力。

[0075] 本文所用关于抗体的术语“竞争”是指第一抗体或其抗原结合部位以充分类似于第二抗体或其抗原结合部位的结合的方式与表位结合，使得第二抗体存在时第一抗体与其关联 (cognate) 表位的结合的结果相较于第二抗体不存在时第一抗体的结合是可检测地降低。或者，该情况可为但不一定是当第一抗体存在时，第二抗体与其表位的结合亦可检测地降低。也就足说，第一抗体可抑制第二抗体与其表位的结合，但第二抗体不抑制所述第一抗体与其相应表位的结合。然而，当各抗体不论以相同、较高或较低程度可检测地抑制另一抗体与其同源表位或配体结合时，所述抗体被称为彼此“交叉竞争”与其相应表位的结合。本发明包含竞争及交叉竞争抗体。不论所述竞争或交叉竞争所藉以发生的机制为何（例如立体阻碍、构象变化、或与共同表位或其部分结合），本领域技术人员将了解根据文中所提供的教示，该竞争和 / 或交叉竞争抗体包含且可用于文中所公开的方法中。

[0076] 本文所用术语“多核苷酸”或“核酸分子”意在包括 DNA 分子及 RNA 分子。核酸分子可以是单链或双链的，但优选是双链 DNA。

[0077] 编码本发明抗体的多核苷酸可以包括以下 :仅变体编码序列，变体编码序列和其他编码序列例如功能性多肽，或信号或分泌序列或前蛋白 (pro-protein) 序列 ;抗体编码序列以及非编码序列，例如内含子或抗体编码序列的 5’和 / 或 3’的非编码序列。术语“编码抗体的多核苷酸”包括多核苷酸，其包括额外的变体编码序列以及包括额外的编码和 / 或非编码序列的多核苷酸。本领域已知为特定宿主细胞 / 表达系统优化的多核苷酸序列可容易地获自期望蛋白的氨基酸序列（参见 GENEART AG, Regensburg, Germany）.

[0078] “宿主细胞”包括可为或已是用于导入多核苷酸插入物的载体的接受者的个别细胞或细胞培养。宿主细胞包括单一宿主细胞的后代，且因为天然、意外或蓄意突变使该后代不一定与原始亲代细胞（在形态学或基因组 DNA 互补性上）完全相同。宿主细胞包括以本发明的多核苷酸在体内转染的细胞。

[0079] 术语“载体”是指构建体，其能在宿主细胞中传送并优选地表达感兴趣的一或多个基因或序列。载体的实例包括但不限于病毒性载体、裸 DNA 或 RNA 表达载体、质体、粘粒或

噬菌体载体、与阳离子缩合剂有关的 DNA 或 RNA 表达载体、包封于脂质体中的 DNA 或 RNA 表达载体及某些真核细胞诸如生产细胞。

[0080] 术语“表达控制序列”是指导核酸转录的核酸序列。表达控制序列可以是启动子，诸如组成型或诱导型启动子或增强子。表达控制序列与待转录的核酸序列可操作连接。

[0081] 编码本发明抗体的多核苷酸通常包括与抗体编码序列可操作连接的表达控制多核苷酸序列，包括本领域已知的天然相关或异源启动子区。优选地，表达控制序列是能转化或转染真核宿主细胞的载体内的真核启动子系统，但也可使用原核宿主细胞的控制序列。一旦载体整合入合适的宿主细胞系，宿主细胞在适合表达核苷酸序列，以及按照需要，适合抗体收集和纯化的条件下繁殖。优选的真核细胞系包括 CHO 细胞系、各种 COS 细胞系、HeLa 细胞、骨髓瘤细胞系、转化的 B 细胞或人胚肾细胞系。最优选的宿主细胞是 CHO 细胞系。

[0082] 抗体

[0083] 本发明的抗体可用本领域熟知的技术生产，例如重组技术、噬菌体展示技术、合成技术或这些技术的组合或其他本领域已知的技术（参见例如 Jayasena, S. D., Clin. Chem., 45:1628-50(1999) 和 Fellouse, F. A. 等人, J. Mol. Biol., 373(4):924-40(2007)）。

[0084] 下表 1 和 2 描述了本发明抗体的优选 CDR。

[0085] 表 1

[0086]

抗体	LCDR1	LCDR2	LCDR3
hu07	TASLSVSSTYLH SEQ ID NO: 14	STSNLAS SEQ ID NO: 15	HQYHRSPLT SEQ ID NO: 16
hu08	KASQDVGTAVA SEQ ID NO: 6	SASYRST SEQ ID NO: 7	QHHYSAPWT SEQ ID NO: 8

[0087] 表 2

[0088]

抗体	HCDR1	HCDR2	HCDR3
hu07	TKYGVH SEQ ID NO: 10	VKWAGGSTDYNSALMS SEQ ID NO: 11	DHRDAMDY SEQ ID NO: 12
hu08	SRNGMS SEQ ID NO: 2	TVSSGGSYIYYADSVKG SEQ ID NO: 3	QGTTALATRFFDV SEQ ID NO: 4

[0089] 本发明的一个实施方案包括抗体或其抗原结合片段，其包含：

[0090] a) 轻链可变区，包含：

[0091] i) 具有选自 SEQ ID NO:6 和 14 的氨基酸序列的 LCDR1；

[0092] ii) 具有选自 SEQ ID NO:7 和 15 的氨基酸序列的 LCDR2；以及

[0093] iii) 具有选自 SEQ ID NO:8 和 16 的氨基酸序列的 LCDR3；以及

[0094] b) 重链可变区，包含：

[0095] i) 具有选自 SEQ ID NO:2 和 10 的氨基酸序列的 HCDR1；

[0096] ii) 具有选自 SEQ ID NO:3 和 11 的氨基酸序列的 HCDR2 ;以及

[0097] iii) 具有选自 SEQ ID NO:4 和 12 的氨基酸序列的 HCDR3。

[0098] 本发明的优选抗体或其抗原结合片段包含 :

[0099] a)LCVR,包含 :SEQ ID NO:6 的 LCDR1, SEQ ID NO:7 的 LCDR2,和 SEQ ID NO:8 的 LCDR3 ;以及

[0100] b)HCVR,包含 :SEQ ID NO:2 的 HCDR1, SEQ ID NO:3 的 HCDR2,和 SEQ ID NO:4 的 HCDR3。

[0101] 本发明的另一优选抗体或其抗原结合片段包含 :

[0102] a)LCVR,包含 :SEQ ID NO:14 的 LCDR1, SEQ ID NO:15 的 LCDR2,和 SEQ ID NO:16 的 LCDR3 ;以及

[0103] b)HCVR,包含 :SEQ ID NO:10 的 HCDR1, SEQ ID NO:11 的 HCDR2,和 SEQ ID NO:12 的 HCDR3。

[0104] 本发明的优选单克隆抗体在本文中被称为 hu08(人源化抗 IL-13-Rα2IgG1 抗体) ;以及 hu07(人源化抗 IL-13-Rα2IgG1 抗体)。编码 mAb hu08 和 hu07 的氨基酸序列的 SEQ ID NO 如下表 3 所示 :

[0105] 表 3

[0106]

mAb	LC	HC	LCVR	LCDR1	LCDR2	LCDR3	HCVR	HCDR1	HCDR2	HCDR3
hu08	51	50	5	6	7	8	1	2	3	4
hu07	53	52	41	14	15	16	48	10	11	12

[0107] 本发明的一个实施方案是抗体或其抗原结合片段,其特异性结合与包含第一氨基酸序列和第二氨基酸序列的抗体相同的 IL-13Rα2 表位,所述第一氨基酸序列与 SEQ ID NO:1 至少 90%、92%、94%、95%、96%、97%、98%或 99%相同,所述第二氨基酸序列与 SEQ ID NO:5 至少 90%、92%、94%、95%、96%、97%、98%或 99%相同。

[0108] 本发明的另一个实施方案是抗体或其抗原结合片段,其特异性结合与包含第一氨基酸序列和第二氨基酸序列的抗体相同的 IL-13Rα2 表位,所述第一氨基酸序列与 SEQ ID NO:48 至少 90%、92%、94%、95%、96%、97%、98%或 99%相同,所述第二氨基酸序列与 SEQ ID NO:41 至少 90%、92%、94%、95%、96%、97%、98%或 99%相同。

[0109] 在一些实施方案中,抗体或其抗原结合片段特异性结合 IL-13Rα2,并且抗体或其抗原结合片段竞争性抑制包含第一氨基酸序列和第二氨基酸序列的抗体的结合,所述第一氨基酸序列与 SEQ ID NO:1 至少 90%、92%、94%、95%、96%、97%、98%或 99%相同,所述第二氨基酸序列与 SEQ ID NO:5 至少 90%、92%、94%、95%、96%、97%、98%或 99%相同。

[0110] 在一些实施方案中,抗体或其抗原结合片段特异性结合 IL-13Rα2,并且抗体或其抗原结合片段竞争性抑制包含第一氨基酸序列和第二氨基酸序列的抗体的结合,所述第一氨基酸序列与 SEQ ID NO:48 至少 90%、92%、94%、95%、96%、97%、98%或 99%相同,所述第二氨基酸序列与 SEQ ID NO:41 至少 90%、92%、94%、95%、96%、97%、98%或 99%相同。

[0111] 本发明的代表性材料于 2012 年 11 月 6 日保藏于美国典型培养物保藏中心 (ATCC)。具有 ATCC 登录号 PTA-13304 的载体是编码命名为 hu08-VLv1.0 的人抗 IL-13 抗体轻链可变区的多核苷酸，具有 ATCC 登录号 PTA-13305 的载体是编码命名为 hu08-VHv1.0 的人抗 IL-13 抗体重链可变区的多核苷酸。这些保藏物是根据国际承认用于专利程序的微生物保存布达佩斯条约（布达佩斯条约）的规定进行。这确保自保藏日开始 30 年内维持该保藏物为活的培养物。该保藏物将依照布达佩斯条约的规定由 ATCC 提供，且将受限于辉瑞 (Pfizer, Inc.) 与 ATCC 的合约，该合约确保当提出相关美国专利或公开任何美国或外国专利申请时（以先到者为主），该保藏物的培养子代可永久且不受限制地供公众使用，且确保由美国专利商标专员依据 35U.S.C. 第 122 节及该专员所依据的法则（包括 37C.F.R. 第 1.14 节，特别参照 886OG638) 确定获得该子代者的可得性。

[0112] 药物部分与抗体的缀合

[0113] 药物部分具有，或经修饰包括可与抗体上的缀合点反应的基团。例如，药物部分可通过烷基化（例如在抗体的 ε - 氨基赖氨酸或 N 末端）、氧化碳水化合物的还原胺化、羟基和羧基之间的酯交换、氨基或羧基的酰胺化以及与硫醇缀合而连接。在一些实施方案中，每个抗体分子缀合的药物部分的数量 p 的平均范围是 1 至 8、1 至 7、1 至 6、1 至 5、1 至 4、1 至 3 或 1 至 2。在一些实施方案中，p 的平均范围是 2 至 8、2 至 7、2 至 6、2 至 5、2 至 4 或 2 至 3。在其他实施方案中，p 的平均数是 1、2、3、4、5、6、7 或 8。在一些实施方案中，p 的平均范围是约 1 至约 8、约 1 至约 7、约 1 至约 6、约 1 至约 5、约 1 至约 4、约 1 至约 3 或约 1 至约 2。在一些实施方案中，p 的平均范围是约 2 至约 8、约 2 至约 7、约 2 至约 6、约 2 至约 5、约 2 至约 4 或约 2 至约 3。对于可用于缀合的化学作用的实例，参见例如 Current Protocols in Protein Science(John Wiley & Sons, Inc.), Chapter 15(Chemical Modifications of Proteins)。

[0114] 接头

[0115] 药物部分可通过接头与抗体连接。适合的接头包括例如可切割和不可切割的接头。可切割的接头通常在胞内条件下易于切割。适合的可切割接头包括例如可被胞内蛋白酶，例如溶酶体蛋白酶或胞内体蛋白酶切割的肽接头。在示例性实施方案中，接头可以是二肽接头，例如缬氨酸 - 瓜氨酸 (val-cit)、苯丙氨酸 - 赖氨酸 (phe-lys) 接头，马来酰亚胺己酰基 - 缬氨酸 - 瓜氨酸 - 对 - 氨基苄氧羰基 (vc) 接头。另一种接头是磺基琥珀酰亚胺 -4-(N- 马来酰亚胺甲基）环己烷 -1- 羧酸酯 (smcc)。磺基 -smcc 缀合通过马来酰亚胺基发生，所述马来酰亚胺基与巯基 (thiols, -SH) 反应，而其磺基 -NHS 酯与伯胺（见于赖氨酸和蛋白或肽的 N 末端）反应。另一种接头是马来酰亚胺己酰基 (mc)。其他适合的接头包括在特定 pH 或 pH 范围可水解的接头，例如腙接头。其他适合的可切割接头包括二硫化物接头。接头可与抗体共价结合，以致抗体必需在胞内降解从而释放药物，例如 mc 接头等等。

[0116] 本发明优选的接头是马来酰亚胺己酰基 - 缬氨酸 - 瓜氨酸 - 对 - 氨基苄氧羰基 (vc) 和马来酰亚胺己酰基 (mc)。

[0117] 改造的 Fc 多肽

[0118] 之前报道据推测存在于抗体重链的 CH2 或 CH3 结构域上，或轻链恒定区上，或其他可接近的某些残基适于用例如半胱氨酸取代天然野生型氨基酸，因此可用于改造能够与各种试剂缀合的位点（参见美国临时专利申请 USSN 61/580169，通过引用并入本文）。

[0119] 氨基酸修饰可通过本领域已知的任意方法进行并且许多这些方法是本领域技术人员熟知并且常规的。例如，但不限于，氨基酸取代、缺失和插入可使用任意熟知的基于PCR的技术实现。氨基酸取代可通过定点诱变进行（参见例如 Zoller 和 Smith, 1982, Nucl. Acids Res. 10:6487-6500；以及 Kunkel, 1985, Proc. Natl. Acad. Sci USA 82:488）。

[0120] 在一些实施方案中，本发明改造的 Fc 多肽可用于制备抗体或其抗原结合片段，这样抗体或其抗原结合片段包含改造的Fc区，其可用于在改造的残基（即与野生型未修饰Fc相比的取代氨基酸）处缀合很多部分。

[0121] 在一些实施方案中，本发明改造的 κ 轻链恒定多肽可用于制备抗体或其抗原结合片段，这样抗体或其抗原结合片段包含改造的 CL 区（其包含氨基酸突变）或其部分，其可用于在改造的氨基酸残基处缀合很多部分。

[0122] 本发明的 IL-13-Rα2 抗体可包括改造的Fc 多肽，其中选自母系、天然或野生型抗体的抗体重链位置 347、392、398、422 和 443 的 1、2 或更多个氨基酸（其中恒定区的编号系统是 Kabat 等人 (1991, NIH Publication 91-3242, National Technical Information Service, Springfield, VA, 以后称为 "Kabat"）中规定的 EU 指数）被其他氨基酸（包括天然和非天然 / 合成氨基酸）取代。

[0123] 应当理解，Fc 多肽中的单取代，例如半胱氨酸残基，通常导致显示所得 IgG 抗体中的两个对应残基，这缘于 IgG 抗体分子的同型二聚体性质。因此，本发明所得改造的 IgG 抗体可显示用于药物或化合物缀合的至少 1、2、3、4 或更多个反应基团。在一个实施方案中，一或多个取代是使用半胱氨酸残基，所得改造的抗体可显示用于药物或化合物缀合的至少 1、2、3、4 或更多个巯基。

[0124] 在其他实施方案中，本发明的改造的 Fc 多肽在选自抗体重链位置 347、392、398、422 和 443 包含一或多个取代，其中恒定区的编号系统是 Kabat 等人中规定的 EU 指数（如前述）。

[0125] 在一些实施方案中，本发明的改造的 Fc 多肽包含至少一对选自以下的氨基酸取代：(a) SEQ ID NO:33 的氨基酸序列；以及 (b) SEQ ID NO:34 的氨基酸序列。

[0126] 在一些实施方案中，本发明的改造的Fc多肽包含一个选自以下的取代：(a) SEQ ID NO:28 的氨基酸序列；以及 (b) SEQ ID NO:29 的氨基酸序列。

[0127] 改造的 Cκ 多肽

[0128] 本发明的 IL-13-Rα2 抗体可以包括改造的抗体轻链恒定区 (LC) 或其部分，其中选自母系、天然或野生型抗体的抗体轻链位置 111、183 或 188 的 1、2 或 3 个氨基酸（其中轻链恒定区的编号系统是Kabat等人 (1991, NIH Publication 91-3242, National Technical Information Service, Springfield, VA, 以后称为 "Kabat"）中规定的 Kabat 编号系统）被其他氨基酸（包括天然和非天然 / 合成氨基酸）取代。。

[0129] 在一些实施方案中，本发明的改造的 LC 多肽包含一或多个选自以下的取代：(a) SEQ ID NO:30 的氨基酸序列；(b) SEQ ID NO:31 的氨基酸序列；以及 (c) SEQ ID NO:32 的氨基酸序列。

[0130] 在其他实施方案中，由于许多抗体的二聚性（例如 IgG 包含两个轻链和两个重链，每个重链包含 Fc 多肽），本发明的抗体可包含至少一个改造的 Fc 多肽，并可还包含至少一个改造的轻链恒定多肽，从而提供至少两个位点特异性缀合位点，一个在 Fc 多肽中，另一

个在 CL 多肽中。本发明优选的抗体（其包含至少一个改造的 Fc 多肽和至少一个改造的轻链恒定区多肽）选自 L443C/kA111C(SEQ ID NO:28 和 30)、L443C/kK183C(SEQ ID NO:28 和 31)、Q347C/kA111C(SEQ ID NO:29 和 30) 以及 Q347C/kK183C(SEQ ID NO:29 和 31)。

[0131] MAC 缀合技术

[0132] 术语多功能抗体缀合物，或 MAC 指本文限定的抗体或其抗原结合部分通过恒定 κ 区共价缀合至至少一个对靶显示生物学效应的药物部分。优选地，抗体或其抗原结合部分包含 SEQ ID NO:52、SEQ ID NO:53、SEQ ID NO:54 或 SEQ ID NO:55 的 K90 和 H91，并且药物部分缀合在 K90。MAC 技术之前在 WO2012/007896 和 USSN 61/584,675 中描述，其通过引用并入本文。

[0133] 药物部分显示对靶的生物学效应，并且可以是肽、小分子、蛋白质、核酸分子、毒素、适配体或抗原结合抗体或其片段。药物部分可以是具有针对靶细胞的细胞毒性的药物。在一些方面，细胞毒素属于一类已知为阿里他汀 (auristatin) 的化合物中。代表性的阿里他汀为本文实施例 13 中所述的化合物 0101 和 3377。

[0134] 药物部分与抗体轻链恒定区的反应特别期望最小化或防止对抗体 Fc 部分与 Fc 受体（例如 FcγR 和 FcRn) 的结合或抗体与其相应靶的结合的任何干扰。相反，相应药物部分与抗体 Fc 部分的缀合可减少抗体体内半衰期和 / 或其与免疫系统（效应物功能）相互作用的能力。药物部分在抗体重链可变区 (VH) 或轻链可变区 (VL) 的缀合有减少抗体与其同源物结合的风险。

[0135] MAC 技术的优势之一是根据试剂和反应条件（尤其是接头抗体的离去基团酯和摩尔比），本发明的组合物和样品可用相对于限定数量的抗体的限定数量的药物部分产生。当平衡药物部分和抗体的相对反应性和治疗窗口时，这尤其有用。而且，在一些情况下，增加每个抗体的药物部分的数量超出某阈值不会导致增加的靶结合或治疗效果。因此能够控制每个抗体缀合的药物部分的数量是有用的，并且由此指导缀合的定位，从而使 Fc 或组合位点干扰最小化。因此，在一些情况下，允许减少缀合的本发明的方面可以是有利的，优选仅修饰单个赖氨酸残基，例如 hu08LC 恒定区的 K90，SEQ ID NO:52、SEQ ID NO:53、SEQ ID NO:54 或 SEQ ID NO:55。另外，尽管缀合至 K90 是可靠并有力的，但缀合至其他抗体表面赖氨酸（每个具有轻微不同的反应性和 pI) 可导致缀合抗体的异质样品，其能在不适合或不定期的时间，例如在流通期间和通过抗体识别将药物部分递送至靶之前释放缀合分子。

[0136] 本发明的另一方面是发现野生型恒定 κ 链 (SEQ ID NO:55)D77 的某些突变改善 K90 位点对药物缀合的易接近性和 / 或反应性。另外，本发明提供 κ 链位置 45 的 V/A 和位置 83 的 A/L 的已知多态性（产生 3 个经鉴定的人恒定 κ 多态性 Km(1):V45/L83、Km(1,2):A45/L83 和 Km(3)A45/V83)。相应地，本发明提供包含 SEQ ID NO:53 的 MAC。在一些方面，本发明提供具有 Km(3) 多态性的 MAC，其中 κ 恒定区选自 SEQ ID NO:52、SEQ ID NO:54 和 SEQ ID NO:55。

[0137] 本发明进一步提供特异性结合人 IL-13Rα2 的抗体，其中所述抗体具有 SEQ ID NO:52 所示的 LC 恒定区，所述 LC 恒定区在位置 80 具有赖氨酸残基 (K80)，并且在位置 77 的丙氨酸残基被天冬氨酸残基取代 (D77A)。

[0138] 本发明进一步提供特异性结合人 IL-13Rα2 的抗体，其中所述抗体具有 SEQ ID NO:53 所示的 LC 恒定区，其中位置 45 是 V 或 A，位置 83 是 A 或 L，位置 77 选自 A、G、I、V、

L、R、S、T、Q、P、N、M、H 和 W。在一些方面，位置 45 是 V，位置 83 是 L。在 SEQ ID NO:53 的一些方面，位置 77 选自 A、G、I、V、L、R、S、T、Q、P、N、M、H 和 W。可对 SEQ ID NO:53 中的位置 45 和 83 的残基可变性进行选择，以便仅提供 Km(1)、Km(1,2) 和 Km(3) 多态性的任意一个、两个或所有三个。

[0139] 本发明进一步提供特异性结合人 IL-13Rα2 的抗体，其中所述抗体具有 SEQ ID NO:54 所示的 LC 恒定区，其中位置 77 选自 A、G、I、V、L、R、S、T、Q、P、N、M、H 和 W。

[0140] 本发明进一步提供特异性结合人 IL-13Rα2 的抗体，其中所述抗体具有 SEQ ID NO:55 所示的 LC 恒定区。

[0141] 癌症治疗

[0142] 癌症，包括但不限于特征在于不可控的细胞生长的肿瘤、转移或其他疾病或病症，可通过施用本发明的抗体 - 药物缀合物进行治疗或预防。

[0143] 示例性抗 IL-13-Rα2 ADC 可用于治疗癌症，其中 IL-13-Rα2 相对于正常（例如非癌组织）表达或过表达。根据本文所述方法，表达 IL-13-RA2 的癌症的治疗或预防可通过向需要该治疗的受试者施用有效量的抗 IL-13-Rα2ADC 来实现。在一些实施方案中，可施用与细胞毒性剂缀合的抗 IL-13-Rα2 全长抗体或其抗原结合片段或其衍生物。在一些示例性实施方案中，本发明的 ADC 会 (i) 结合表达 IL-13-Rα2 的癌细胞，以及 (ii) 显示细胞毒性或细胞生长抑制效应，例如抑制表达 IL-13-Rα2 的癌细胞的增殖，或杀死表达 IL-13-RA2 的癌细胞。

[0144] 在其他实施方案中，抗 IL-13-Rα2 ADC 与其他治疗剂共同施用，或与其他治疗剂先后施用。在一些实施方案中，抗 IL-13-Rα2 ADC 与化疗药物，包括标准治疗化疗药物共同施用，或先后施用。

[0145] 在一些实施方案中，其他治疗剂可以是待治疗的特定疾病的标准治疗制剂，或是作为待治疗的特定疾病的救助方案一部分的制剂。抗癌剂和化疗方案包括例如抗癌抗体，其包括例如抗 CD52 抗体（例如阿仑单抗）、抗 CD20 抗体（例如利妥昔单抗）以及抗 CD40 抗体（例如 SGN40）；化疗方案，其包括例如 CHOP（环磷酰胺、阿霉素、长春花新碱和强的松）；CVP（环磷酰胺、长春花新碱和强的松）；RCVP（利妥昔单抗 +CVP）；RCHOP（利妥昔单抗 +CHOP）；RICE（利妥昔单抗 + 异环磷酰胺、脱羧铂氨、依托泊苷）；RDHAP（利妥昔单抗 + 地塞米松、阿糖胞苷、顺式铂氨）；RESHAP（利妥昔单抗 + 依托泊苷、甲泼尼龙、阿糖胞苷，顺式铂氨）；吉西他滨；长春花新碱、强的松和葸环类的组合治疗，有或无门冬酰胺酶；柔红霉素、长春花新碱、强的松和门冬酰胺酶的组合治疗；替尼泊苷和 Ara-C（阿糖胞苷）的组合治疗；氨甲蝶呤和甲酰四氢叶酸的组合治疗；博来霉素、阿霉素、依托泊苷、氮芥、强的松、长春花碱和长春花新碱的组合治疗；小分子抑制剂；以及蛋白酶抑制剂包括例如硼替佐米。

[0146] 在一些实施方案中，治疗癌症的方法包括向需要的患者施用有效量的抗 IL-13-Rα2 ADC 并联合放疗，以及任选地其他治疗剂。在一些实施方案中，抗 IL-13-Rα2 ADC 与抗癌剂（例如化疗剂）和 / 或放疗共同或先后施用。在一些实施方案中，化疗剂或放疗在施用本发明的化合物之前或之后至少 1 小时、5 小时、12 小时、1 天、1 周、1 个月、几个月（例如多达 3 个月）施用。

[0147] 本发明的 ADC 可以是用于施用的药物组合物形式（其配制为适于选择的施用模式）以及药物学可接受的稀释剂或赋形剂形式，例如缓冲液、表面活性剂、防腐剂、增溶剂、

等渗剂、稳定剂、载体等等。Remington's Pharmaceutical Sciences,Mack Publishing Co.,Easton Pa.,18th ed.,1995 提供成型工艺的概要,其为医师普遍知晓。

[0148] 这些药物组合物可通过本领域已知的实现癌症治疗的一般目的的任意方式施用。优选的施用途径是不经肠道的,本文指包括但不限于以下的施用模式:静脉内、肌内、腹膜内、皮下和节间注射及灌注。施用剂量将取决于受者的年龄、健康和体重,同步治疗种类(如果有),治疗频率和期望效果的性质。

[0149] 本发明范围内的组合物包括所有组合物,其中 ADC 以有效获得癌症治疗的期望的医疗效果的量存在。由于不同患者的个体需求会有差异,确定所有成分的有效量的最佳范围是具有普通技术的临床医生的能力范围。

[0150] 诊断

[0151] 本发明的抗体或抗体片段也可用于体外或体内检测生物样品中的 hIL-13-Rα2。在一个实施方案中,本发明的抗 hIL-13-Rα2 抗体用于确定组织或来自组织的细胞中的 hIL-13-Rα2 水平。在一个优选实施方案中,所述组织是疾病组织。在一个优选的方法实施方案中,所述组织是肿瘤或其活组织。在一个优选的方法实施方案中,组织或其活组织先从患者切除,然后可用本发明的抗体或抗体片段在免疫测定中确定组织或活组织中的 hIL-13-Rα2 水平。组织或其活组织可冷冻或固定。相同的方法可用于确定 hIL-13-Rα2 蛋白的其他性质,例如其细胞表面水平,或细胞定位。

[0152] 上述方法可用于诊断已知或疑似患有癌症的受试者的癌症,其中所述患者中测量的 hIL-13-Rα2 水平与正常参考受试者或标准比较。所述方法可用于确定肿瘤是否表达 hIL-13-Rα2,其可表明该肿瘤会对本发明的抗体 - 药物缀合物治疗反应良好。优选地,肿瘤是肺癌、结肠癌、胃癌、胰腺癌、卵巢癌、恶性胶质瘤和黑素瘤癌症,或 hIL-13-Rα2 过表达的其他癌,以及其中 hIL-13-Rα2 显著表达的有待确定的其他癌症。

[0153] 本发明的一个实施方案是治疗表达 IL-13-Rα2 的癌症的方法,所述方法包含:确定生物样品中的 hIL-13-Rα2 水平,其包含以下步骤:获得来自疑似患有癌症的受试者的样品;使用本发明的抗体在免疫测定中测试所述样品;确定所述样品上的 hIL-13-Rα2 的细胞表面水平;将 hIL-13-Rα2 的细胞表面水平与正常参考受试者或标准水平比较;以及向所述受试者施用本发明的抗体 - 药物缀合物。

[0154] 本发明进一步提供单克隆抗体、人源化抗体及其表位结合片段,其进一步标记用于研究或诊断应用。在优选实施方案中,所述标记是放射性标记、荧光团、生色团、显像剂或金属离子。

[0155] 还提供诊断方法,其中所述标记抗体或其表位结合片段施用于疑似患有癌症的受试者,并测量或监测标记在受试者体内的分布。

[0156] 试剂盒

[0157] 本发明还包括试剂盒,例如包含描述的细胞毒性缀合物和使用细胞毒性缀合物杀死特定细胞类型的说明书。说明书可包括在体外、体内或离体使用细胞毒性缀合物的用法说明。通常地,试剂盒会有含有细胞毒性缀合物的区室。细胞毒性缀合物可以是低压冻干形式、液体形式或可经调整包括在试剂盒中的其他形式。试剂盒也可含有按照试剂盒中的说明书执行所述方法所需的额外元件,例如复溶低压冻干粉末的灭菌溶液,在向患者施用前与细胞毒性缀合物组合的额外试剂,以及辅助向患者施用缀合物的工具。

[0158] 之前或后续实施例中引用的所有出版物和专利文献通过引用整体并入本文，如同每个单独表示。

[0159] 本发明将参考后续实施例进一步描述，然而，应当理解的是，本发明不限于这些实施例。

[0160] 用于执行本发明的特定方面的后续实施例仅出于示例性目的提供，无意于以任意方式限制本发明的范围。

[0161] 实施例 1

[0162] 鼠抗 IL-13Rα2 抗体 mu07 和 mu08 的产生和评估

[0163] 在小鼠中用人 IL-13Rα2 抗原和免疫标准方法制备抗 hIL-13Rα2 抗体。(Zhang,C.,Antibody Methods and Protocols,Methods in Molecular Biology,vol.901,DOI 10.1007/978-1-61779-931-0_7, © Springer Science+Business Media,LLC 2012)。两种鼠抗体 mu07 和 mu08 经鉴定结合 A375 细胞，一种黑素瘤细胞系，其在细胞表面内源表达高水平的 IL-13Rα2。

[0164] ADC 中抗体的一个重要特征是与其受体结合后快速内化。在与 A375 细胞在 37℃孵育 1 小时后，对抗体 mu07 和 mu08 进行评估并发现其分别内化 38％和 31％。

[0165] 实施例 2

[0166] 鼠抗 IL-13Rα2 抗体 mu07 和 mu08 的可变区

[0167] 使用 SMARTer® cDNA 合成系统 (Clontech Laboratories Inc.of Mountain View,Calif.) 克隆 mu07 和 mu08 抗 IL-13Rα2 抗体重链和轻链可变区，随后经 PCR 扩增。通过标准技术合成 cDNA，并使用与 SMARTer® IIA 寡核苷酸序列退火的引物和小鼠恒定区特异性引物（小鼠 κ 轻链和小鼠 IgG1 重链）以及 PCR SuperMix High Fidelity(Invitrogen,Carlsbad,CA.)，经 PCR 扩增 cDNA。重链和轻链可变区 PCR 产物亚克隆入 pCR4-TOPO 载体 (Invitrogen,Carlsbad,CA)，并确定核酸序列。

[0168] mu07 和 mu08 重链可变区的氨基酸序列分别如 SEQ ID NO:25 的氨基酸残基和 SEQ ID NO:23 的氨基酸残基所示。mu07 和 mu08 轻链可变区的氨基酸序列分别如 SEQ ID NO:26 和 SEQ ID NO:24 所示。

[0169] 实施例 3

[0170] 嵌合抗体 ch07 和 ch08 的结合特异性和结合动力学

[0171] 使用本领域已知的方法构建嵌合抗体 07 和 08(ch07 和 ch08)，其具有鼠重链和轻链可变区序列与人 IgG1 重链恒定区和人 κ 轻链恒定区。为了评估 ch07 和 ch08 的结合活性和特异性，使用重组 hIL-13-Rα2 和 hIL-13Rα1(IL-13 细胞因子受体）进行标准直接 ELISA 方案。通过辣根过氧化物酶 (HRP) 缀合的山羊抗人 IgGKappa 检测结合。图 1 的结果表明两种嵌合抗体都能特异性结合 hIL-13Rα2，但不结合 hIL-13Rα1。ch07 和 ch08 的 ED50 分别是 0.15nM 和 0.076nM。

[0172] 为了评估 ch07 和 ch08 抗体的结合动力学，在 Biacore® T100 或 T200 装置上使用 Biacore® 人 Fab 捕获试剂盒 (GE Healthcare) 进行 SPR（表面等离子体共振）实验。使用 Biacore® T100 评估软件版本 2.0，以 1:1 Langmuir 结合模型分析所有数据。

[0173] K_a、K_d 和 KD 示于表 5。pH7.4 时，ch07 和 ch08 与 hIL-13-Rα2 的结合亲和力均在

pM 范围内，分别为 648pM 和 964pM。ch07 从 hIL-13-Rα2 上的解离比 ch08 慢约 2 倍。pH6.0 时，ch07 和 ch08 与 hIL-13-Rα2 的结合亲和力均在低 nM 范围内。ch07 和 ch08 的解离速率非常相似。pH6.0 时比 pH7.4 时的 KD 更高缘于较慢的结合速率。

[0174] 表 5 嵌合抗体 ch07 和 ch08 的动力学分析

[0175]

	Ka(1/Ms)	Kd(1/s)	KD(M)
ch07-pH 7.4	3.61E+05	2.34E-04	6.48E-10
ch07-pH 6.0	6.43E+04	3.37E-04	5.24E-9
ch08-pH 7.4	4.32E+05	4.16E-04	9.64E-10
ch08-pH 6.0	7.12E+04	3.81E-04	5.36E-9

[0176] 实施例 4

[0177] 抗体 ch07 和 ch08 的结合表位

[0178] 进行竞争 ELISA 以检测 ch07 和 ch08 是否具有不同的结合表位。在竞争 ELISA 实验之前，ch07 和 ch08 被生物素化。通过直接标准 ELISA 确定生物素化的 ch07(biotin-ch07) 和 ch08(biotin-ch08) 的 EC50。对于竞争 ELISA，将位于 PBS 中的 50ul 2μg/mL 重组 hIL-13-Rα2 于 4℃过夜包被在 96 孔板上。然后按照标准 ELISA 方案封闭并清洗平板。3 倍系列稀释的 ch07 和 ch08(2x 终浓度）分别与恒定量的 biotin-ch07（图 2A) 或 biotin-ch08（图 2B) 混合，并添加到平板，室温孵育 1 小时。通过 1:5000 的 HRP 缀合的链霉亲和素经 1 小时检测生物素化的嵌合抗体的结合量。结果如图 2 所示。未标记的嵌合 ch07 与 biotin-ch07 竞争结合 hIL-13-Rα2，而未标记的 ch08 未显示竞争迹象（图 2A)。当相同设置的抗体用于与 biotin-ch08 竞争时，获得相似的结果（图 2B)。这明确证实抗体 ch07 和 ch08 具有不同的 IL-13Rα2 结合表位。

[0179] 还用两种商业化可购的抗体，单克隆小鼠 IgG1，MAB614(R&D Systems) 和单克隆小鼠 IgG1，ab27414(Abcam) 进行竞争 ELISA。图 2A 和图 2B 显示，两种商业化抗体均不与 biotin-ch07 或 biotin-ch08 竞争结合 IL-13Rα2，表明抗体 ch07 和 ch08 具有与两种商业化抗体不同的结合表位。

[0180] 该结果经 BiaCore 实验确证。使用胺偶联化学反应将约 100RUhIL-13-Rα2 固定在 CM5 芯片上。ch07(100nM 和 200nM) 和 ch08(100nM 和 200nM) 以 10ul/min 的流速经 150s 先后通过 hIL-13-Rα2 实验通道和对照通道注射。当 100nM ch07 注射入固定的 hIL-13Rα2 时，达到 50RU。当 ch07 浓度增加至 200nM 时，RU 不再增加，表明 hIL-13-Rα2 上的 ch07 结合位点饱和。当注射入 100nM ch08 时，增加 100RU。该阳性结合信号表明两种抗体缺乏竞争。结果进一步确证抗体 ch07 和 ch08 具有不同的结合表位（图 2C)。

[0181] 实施例 5

[0182] ch07 和 ch08 中和研究

[0183] 为了评估 ch07 和 ch08 能否中和 IL-13 的功能，进行竞争 ELISA。抗 Flag 抗体包被于 96 孔板上并 4℃孵育过夜。然后按照标准 ELISA 方案封闭并清洗平板。3 倍系列稀释

的小鼠抗体、嵌合抗体、阳性对照裸 IL-13Rα2 和阴性对照 hIL-21R 与恒定量的生物素化 IL-13Rα2-Fc(4x ED50) 和恒定量的 IL-13(4x ED50) 在室温下孵育 1 小时。100ul 复合物添加至 ELISA 板，并在室温孵育 1 小时。通过 1:5000 的 HRP 缀合的链霉亲和素经 1 小时检测 biotin-IL-13-Rα2 的结合量。结果如图 3 所示。抗体 07 和 08（鼠和嵌合形式）均不与 IL-13 竞争 IL-13-Rα2 结合位点，而裸 IL-13Rα2 与生物素化 IL-13Rα2 竞争。这表明抗体 07 和 08（鼠和嵌合形式）是非中和性抗体。

[0184] 实施例 6

[0185] mu08 人源化

[0186] 使用 DP-54 和 DPK9 作为人受体框架对单克隆鼠抗体 mu08(Seq ID NO:23 和 24) 进行人源化。通过有或无回复突变的 CDR 移植制备人源化 08 抗体 (hu08)。使用 Kabat 方案鉴定鼠 mu08 抗体的 CDR。

[0187] 通过将 mu08 的 CDR 直接移植到人 DP-54 框架区上，构建 hu08 重链可变区 (VH 版本 1.0)。通过在框架 DP-54 的位置 A40T、G42D、G44R 和 N83S 处的回复突变制备版本 1.1。v1.0 和 v1.1 均克隆入含有 hIgG1 恒定区的 pSMED2 载体。编码人源化 hu08 重链可变区的核苷酸序列是 v1.0 的 SEQ ID NO:17 和 v1.1 的 SEQ ID NO:20。编码 hu08 重链可变区的氨基酸序列是 v1.0 的 SEQ ID NO:1 和 v1.1 的 SEQ ID NO:19。

[0188] 通过将鼠 mu08 的 CDR 直接移植到人 DPK9 框架区上，构建 hu08 轻链可变区 (VK 版本 1.0)。通过在框架区 DPK9 的位置 K39I、S60D、T72S、T73F 和 T74I 的回复突变制备版本 1.1。v1.0 和 v1.1 均克隆入含有 hIgKappa 恒定区的 pSMEN3 载体。编码 hu08 轻链可变区的核苷酸序列是 v1.0 的 SEQ ID NO:18 和 v1.1 的 SEQ ID NO:22。编码 hu08 轻链可变区的氨基酸序列是 v1.0 的 SEQ ID NO:5 和 v1.1 的 SEQ ID NO:21。

[0189] 实施例 7

[0190] 人源化 hu08 的表征

[0191] 通过标准直接 ELISA 评估与重组 hIL-13-Rα2 结合的人源化 hu08。重组 hIL-13-Rα2 包被于 96 孔板上。系列稀释的与版本 1.0 和 1.1 的重链和轻链组合的嵌合抗体或人源化抗体，例如 hu08 v1.0HC/1.0LC、hu08 v1.0HC/v1.1LC、hu08 v1.1HC/v1.0LC 和 hu08 v1.1HC/v1.1LC 添加至平板并在室温下孵育 1-2 小时。通过 HRP 缀合的山羊抗人 Ig Kappa 检测结合。结果如下表 6 所示，其表明所有四种人源化抗体的组合均能结合重组 hIL-13-Rα2，并且 ED50 与 ch08 类似。

[0192] 进行标准 FACS（荧光激活细胞分选）分析以评估抗体与细胞表面 IL-13Rα2 的结合活性。用含 1％牛血清白蛋白和 0.001％叠氮化钠的冰冷的 PBS 清洗 A375 细胞。用系列稀释的抗体 hu08 v1.0 和 hu08 v1.1 例如 hu08v1.0HC/1.0LC、hu08 v1.0HC/v1.1LC、hu08 v1.1HC/v1.0LC 和 hu08 v1.1HC/v1.1LC 于 4℃孵育细胞 30 分钟，然后用磷脂酰乙醇胺标记的山羊抗人 IgG 染色，在含 4％多聚甲醛的 PBS 中固定，并在 FACScan® (BD Biosciences) 上分析。数据与和重组受体的结合一致并表明所有 4 种组合与细胞表面抗原的结合与 ch08 类似（表 6）。

[0193] 进行竞争 ELISA 以评估 hu08 1.0 和 hu08 v1.1 是否与 ch08 竞争结合 IL-13-Rα2。将 PBS 中的 50ul 2μg/mL 重组 hIL-13-Rα2 于 4℃过夜包被在 96 孔板上。然后按照标准 ELISA 方案封闭并清洗板。3 倍系列稀释的 ch08、hu08 1.0 和 hu08 v1.1 以及阴性对照

ch07(2x 终浓度）与生物素化的 ch08(2x EC_{50}) 混合。50 μ l 抗体和 biotin-ch08 的混合物添加至平板并在室温下孵育 1 小时。通过 HRP 缀合的链霉亲和素检测结合的 biotin-ch08 量。结果如表 6 所示。所有 4 种人源化抗体的组合类似于嵌合抗体 ch08，与生物素化 ch08 竞争，表明 hu08 1.0 和 hu08 v1.1 保留与 ch08 相同的结合表位并具有与可溶和细胞表面 IL-13-Rα2 相似的亲和力。

[0194] 表 6

[0195]

抗体	EC50 (nM) 重组 hIL-13Rα2	EC50 (nM) A375 细胞	IC50 (nM) Biotin-ch08
ch08Hc+Lc	0.152	5.637	1.979
hu08 v1.0/1.0	0.175	4.098	2.120
hu08 v1.0/1.1	0.143	6.522	2.144
hu08 v1.1/1.0	0.163	4.152	2.562
hu08 v1.1/1.1	0.198	5.157	3.164

[0196] hu08 v1.0HC/v1.0LC 和 hu08 v1.1HC/v1.1LC 经扩增产生纯化蛋白。两种抗体均在 HEK293F 悬浮细胞中瞬时表达。令人惊讶的是，与 ch08 产量相比，08 的人源化使抗体产量提高了 5-6 倍（表 7）。

[0197] 表 7

[0198]

抗体名称	表达水平 (ug/ml)
ch08	15
hu08v1.0/1.0	89.6
hu08v1.1/1.1	72.3

[0199] 蛋白或蛋白结构域的热稳定性与蛋白或蛋白结构域的整体稳定性之间存在直接相关。蛋白或蛋白结构域的较高熔点经常提供改善的可制造性和更长的储藏期限 / 稳定性。通过差示扫描量热法 (DSC) 检测 ch08 和 hu08(v 1.0 和 v1.1) 的热稳定性。使用标准方案在 MicroCal VP-DSC 装置上进行 DSC 引起的嵌合和人源化抗体的热解折叠。两种人源化抗体 hu08 v1.0/1.0 和 hu08 v1.1/1.1 均显示比嵌合版本 ch08 较高的 Tm2(Fab)（表 8）。这表明 cu08 的人源化改善该抗体的热稳定性。

[0200] 表 8

[0201]

	CH2	Fab	CH3
抗体	Tm1 (℃)	Tm2 (℃)	Tm3 (℃)
ch08	73.41+0.47	70.44+0.04	84.35+0.09
hu08v1.0/1.0	73.48±0.19	80.29±0.02	85.48±0.14
hu08v1.1/1.1	71.12+0.13	80.23+0.02	

[0202] 在 Biacore® T100 上如上所述进行 hu08 抗体的结合动力学，结果示于表 9。

[0203] 表 9

[0204]

抗体	抗原	pH	ka(1/Ms)on	kd(1/s)off	KD(nM)
hu08 v1.0	hIL-13Rα2	7.4	9.75E+04	2.46E-04	2.52E-09
hu08 v1.0	hIL-13Rα2	6.0	5.29E+04	2.34E-04	4/42E-09

[0205] 实施例 8

[0206] mu07 人源化

[0207] 使单克隆鼠抗体 mu07 人源化的总体策略与实施例 6 中所述的 mu08 的策略相同。通过将 mu07 的 CDR 直接移植到人 DP-54 框架区上，构建人源化 hu07 重链可变区 (VH 版本 1.0)。通过在框架 DP-54 的不同位置的回复突变制备版本 1.1-1.5。所有版本均克隆入含有 hIgG1 恒定区的 pSMED2 载体。

[0208] 通过将 mu07 的 CDR 直接移植到人 DPK9 框架区上，构建 hu07 轻链可变区 (VK 版本 1.0)。通过在框架 DP-54 的不同位置的回复突变制备版本 1.1-1.7(表 10)。所有版本均克隆入含有 hIg Kappa 恒定区的 pSMEN3 载体。编码 hu07 可变区的氨基酸序列的 SEQ ID 号如表 10 所列。

[0209] 表 10

[0210]

	hu07 VH		hu07 VK		
VH 变体	aa SEQ ID	回复突变	VL 变体	aa SEQ ID	回复突变
hu07 VH v1.0	9		hu07 VK v1.0	13	
hu07 VH v1.1	37	T28S, F29L, A49G, F67L, N76S	hu07 VK v1.1	42	K41S, A42S, D70S
hu07 VH v1.2	38	R71K	hu07 VK v1.2	43	L47W
hu07 VH v1.3	39	T28S, F29S, R71K	hu07 VK v1.3	44	F71Y
hu07 VH v1.4	40	T28S, F29S,	hu07 VK v1.4	45	L47W, F71Y
hu07 VH v1.5	41	T28S, F29S, A49G, R71K	hu07 VK v1.5	46	K41S, A42S, D70S, L47W
			hu07 VK v1.6	47	K41S, A42S, D70S, F71Y
			hu07 VK v1.7	48	K41S, A42S, D70S, L47W, F71Y

[0211] 实施例 9

[0212] 人源化 hu07 的表征

[0213] 为了评价各种版本 hu0 的结合 / 竞争性能，用 19 种 hu07 重链和轻链的组合在 COS-1M6 细胞中进行瞬时转染。包括 6 种重链和 3 种轻链 :嵌合重链，人源化 v1.0-1.5 重链，嵌合轻链和人源化 v1.0-1.1 轻链。转染后 2 天收获条件培养基 (CM) 并使用本领域已知的方案，通过标准 ELISA 进行与重组 IL-13-Rα2 的直接结合，通过基于细胞的 ELISA（使用 A375 细胞）进行细胞表面受体结合，以及与生物素化 ch07 的竞争 ELISA。

[0214] 基于来自 CM 的最初筛选数据，选择重链 v1.5 进行进一步研究。重链 v1.5 与嵌合、人源化 v1.0 和 v1.1 轻链配对。表 11 总结了 hu07 抗体的结合活性、竞争性能和细胞毒性。与嵌合轻链 Kc 配对的 hu07v1.5 显示与嵌合抗体相似的 ED50 和 IC50。当该重链 v1.5 与轻链 v1.0 和 v1.1 配对时，在 A375 细胞上的竞争活性和重组蛋白减少。

[0215] 表 11

[0216]

	IC50 (nM) 竞争 ELISA 平板	**ED50 (nM) ELISA rhIL-13Rα2**	**ED50 (nM) cELISA A375 细胞**	**IC50 (nM) 竞争 ELISA A375 细胞**	**IC50 (nM) Saporin 测定 A375 细胞**
ch07	2.21	0.217	0.85	17.27	0.08
hu07v1.5/kc	3.03	0.183	0.87	7.67	ND
hu07v1.5/v1.0	62.00	0.754	1.17	12.27	0.19
hu07v1.5/v1.1	46.62	1.214	1.29	19.00	0.20

[0217] hu07 重链 v1.5 用于轻链优化。在 COS-1M6 细胞中进行 10 种 hu07 重链和轻链组合的瞬时转染。重链 v1.5 与嵌合轻链和人源化版本 v1.0-1.7 配对。收获 CM 并用于实验以通过 ELISA 确定与重组 hIL-13α2(rhIL-13α2) 的结合亲和力，并通过竞争 ELISA 确定与生物素化 ch07 的竞争活性。数据表明重链 v1.5 和轻链 v1.7 的组合最优。用纯化蛋白确证该结果（表 12)。

[0218] 表 12

[0219]

	ED50(nM)rhIL-13Rα2	IC50(nM) 竞争 ELISA 平板
ch07	0.39	2.21
hu07Hv1.5/kv1.7	0.39	7.7

[0220] 实施例 10

[0221] ch07、hu07、ch08 和 hu08 的种属交叉反应性

[0222] 通过 RT-PCR 从猕猴 (cyno) 睾丸和脂肪组织分离猕猴 IL-13-Rα2（胞外结构域 (ECD)）和跨膜结构域 (TM)。猕猴 IL-13-Rα2 的氨基酸序列如 SEQ ID NO:27 所示。人和猕猴 IL-13Rα2 之间的相同性为 94％。

[0223] 将与 Flag 标签在 C 末端融合的猕猴 IL-13Rα2 ECD/TM 结构域克隆入 pSMED2 表达载体。用含有 cyno-IL-13-Rα2 的质粒和 pSMED2 载体（作为转染试剂对照）瞬时转染 HEK293 悬浮细胞。72 小时后收获细胞并进行 FACS 分析。测试 4 种抗体，包括 ch07、ch08、

hu08v1.0/1.0 和 hu08v1.1/1.1。用藻红蛋白标记的山羊抗人或小鼠 IgG 检测在细胞表面 cyno-IL-13-Rα2 上的结合。数据显示 ch07、ch08、hu08v1.0/1.0 和 hu08v1.1/1.1 能够与细胞表面 cyno-IL-13-Rα2 结合并具有相似的结合亲和力 (ED50)(表 13)。

[0224] 表 13

[0225]

抗体	ED50(nM)
ch07	1.494
ch08	1.814
hu08v1.0/1.0	1.961
hu08v1.1/1.1	2.141

[0226] 通过直接 ELISA 评估 hu07 和 hu08 与小鼠 IL-13-Rα2 的结合。人和小鼠 IL-13-Rα2 氨基酸水平的相同性约为 64%。重组 mIL-13-Rα2 或 hIL-13-Rα2(作为阳性对照)包被于 96 孔板上。纯化的嵌合 ch07 和 ch08 经系列稀释并添加于抗原包被的平板。通过 HRP 缀合的山羊抗人 IgGFc 特异性二抗检测结合的抗体。mIL-13-Rα2 结合无可检测信号,而与阳性对照 hIL-13-Rα2 的结合强烈,表明 hu07 和 hu08 不与鼠 mIL-13Rα2 交叉反应。

[0227] 实施例 11

[0228] 与表达 IL13R-α2 的人细胞系的结合

[0229] 将表达 IL13R-α2 抗原的细胞系和阴性对照细胞以每孔 200,000 个细胞的密度铺于 96 孔深孔板并保持在冰上。将在含 3%牛血清白蛋白 BSA 的杜氏磷酸盐缓冲液 (DPBS) 中的制备的小鼠单克隆抗体 mu07 或 mu08 添加至平板,终浓度 10μg/mL。然后将平板在冰上孵育 1 小时,随后清洗 2 次。将 PE(藻红蛋白)缀合的山羊抗小鼠 IgG Fc 二抗添加至孔中。4℃孵育 30 分钟后,继而在 FACScan™(BD Biosciences) 上通过 FACS 分析平均荧光强度。

[0230] 表 14 中的数据表明 mu07 和 mu08 抗体与来自不同疾病迹象的不同组 IL-13R-α2 阳性细胞系结合。

[0231] 表 14

[0232]

人细胞系	平均荧光强度		IL-13-Rα2 表达
	mu07	mu08	
PC3MM2 (前列腺)	84000	72000	3+
U87MG (恶性胶质瘤)	61000	62000	3+
A375 (黑素瘤)	53000	46000	3+
H460 (肺，顺式铂氨抗性)	28000	22000	2+
Hs766T (胰腺)	13000	14000	2+
A498 (肾)	13000	5000	1+
SW626 (卵巢)	9000	8000	1+
H460 (肺)	800	300	0

[0233] 实施例 12

[0234] 内化

[0235] 抗体内化是在表达 IL-13-Rα2 的细胞中递送 ADC 细胞毒性的关键特征。使用 mu07 和 mu08 抗体以及阳性对照小鼠单克隆抗体 (ab27414) 在 4 种细胞系 (PC3MM2、A375、Hs766T 和 H460R) 上检测抗体与 IL-13-Rα2 结合后的内化。抗体 (10μg/mL) 与不同细胞在冰上孵育 1 小时，通过用冷培养基清洗两次，去除未结合的抗体。于 37℃孵育细胞培养平板。细胞样品固定 15 分钟和 4 小时。不同时间点的内化百分比如表 15 所示。数据表明 mu07 和 mu08 易于内化入表达 IL-13-Rα2 的细胞。

[0236] 表 15

[0237]

内化%								
一抗	15 分钟				4 小时			
	A375	Hs766T	PC3MM2	H460R	A375	Hs766T	PC3MM2	H460R
mu07	63.1	69.6	88.6	74.4	38.1	69.0	50.3	38.4
mu08	68.0	64.1	81.9	55.9	61.8	76.3	41.6	47.4
ab27414	49.3	60.8	60.2	57.5	68.2	73.6	49.7	49.2

[0238] 实施例 13

[0239] 化合物 0101 和 3377 的合成

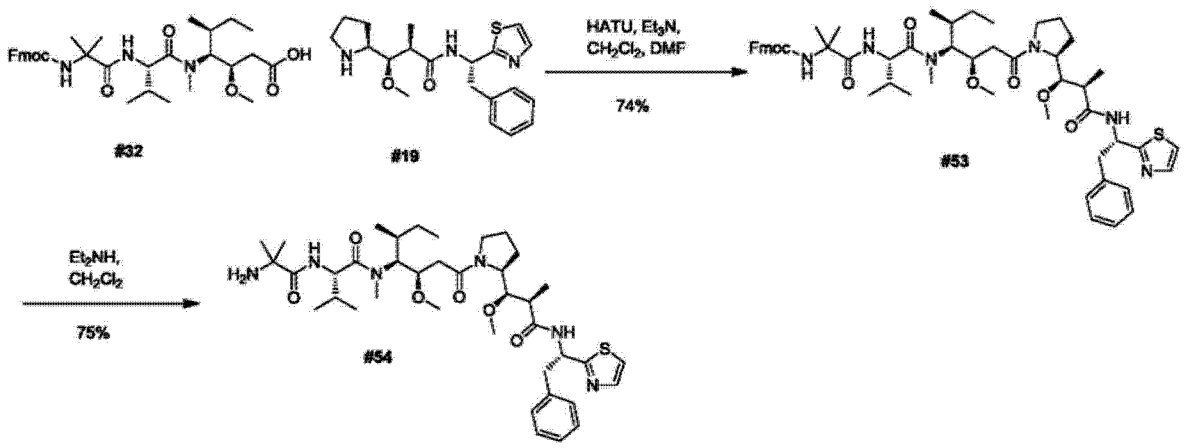
[0240] 根据美国专利申请 13/670,612 描述的方法制备化合物 0101 和 3377，其通过引用并入本文。

[0241] 化合物 0101 的实验 (示意图 #54)

[0242] 2- 甲基丙氨酰 -N-[(3R,4S,5S)-3- 甲氧基 -1-{(2S)-2-[(1R,2R)-1- 甲氧基 -2- 甲基 -3- 氧 -3-{[(1S)-2- 苯基 -1-(1,3- 噻唑 -2-yl) 乙基] 氨基 } 丙基] 吡咯

烷 -1-yl}-5- 甲基 -1- 氧代庚烷 -4-yl]-N- 甲基 -L- 缬氨酰胺 (#54) 的制备

[0243]



[0244] 步骤 1、N-[(9H- 芴 -9-yl 甲氧基) 羰基]-2- 甲基丙氨酰 -N-[(3R,4S,5S)-3- 甲氧基 -1-{(2S)-2-[(1R,2R)-1- 甲氧基 -2- 甲基 -3- 氧 -3-{[(1S)-2- 苯基 -1-(1,3- 噻唑 -2-yl) 乙基] 氨基 } 丙基] 吡咯烷 -1-yl}-5- 甲基 -1- 氧代庚烷 -4-yl]-N- 甲基 -L- 缬氨酰胺 (#53) 的合成。根据一般程序 D,利用二氯甲烷 (20mL,0.1M) 和 N,N- 二甲基酰胺 (3mL) 中的 #32(2.05g,2.83mmol,1eq.)、胺 #19(2.5g,3.4mmol,1.2eq.)、HATU(1.29g,3.38mmol,1.2eq.) 和三乙胺 (1.57mL,11.3mmol,4eq.) 合成粗制的期望的材料,通过硅胶层析 (梯度 0% 至 55% 位于庚烷中的丙酮) 纯化,产生固体 #53(2.42g,74%)。LC-MS :m/z 965.7[M+H+],987.6[M+Na+],保留时间 = 1.04 分钟 ;HPLC(方案 A) :m/z 965.4[M+H+],保留时间 = 11.344 分钟 (纯度 >97%) ;1H NMR(400MHz,DMSO-d6),假定为旋转异构体混合物,特征信号 :δ 7.86-7.91(m,2H),[7.77(d,J = 3.3Hz) 和 7.79(d,J = 3.2Hz),总 1H],7.67-7.74(m,2H),[7.63(d,J = 3.2Hz) 和 7.65(d,J = 3.2Hz),总 1H],7.38-7.44(m,2H),7.30-7.36(m,2H),7.11-7.30(m,5H),[5.39(ddd,J = 11.4,8.4,4.1Hz) 和 5.52(ddd,J = 11.7,8.8,4.2Hz),总 1H],[4.49(dd,J = 8.6,7.6Hz) 和 4.59(dd,J = 8.6,6.8Hz),总 1H],3.13,3.17,3.18 和 3.24(4s,总 6H),2.90 和 3.00(2br s,总 3H),1.31 和 1.36(2br s,总 6H),[1.05(d,J = 6.7Hz) 和 1.09(d,J = 6.7Hz), 总 3H]。

[0245] 步骤 2、2- 甲基丙氨酰 -N-[(3R,4S,5S)-3- 甲氧基 -1-{(2S)-2-[(1R,2R)-1- 甲氧基 -2- 甲基 -3- 氧 -3-{[(1S)-2- 苯基 -1-(1,3- 噻唑 -2-yl) 乙基] 氨基 } 丙基] 吡咯烷 -1-yl}-5- 甲基 -1- 氧代庚烷 -4-yl]-N- 甲基 -L- 缬氨酰胺 (#54) 的合成

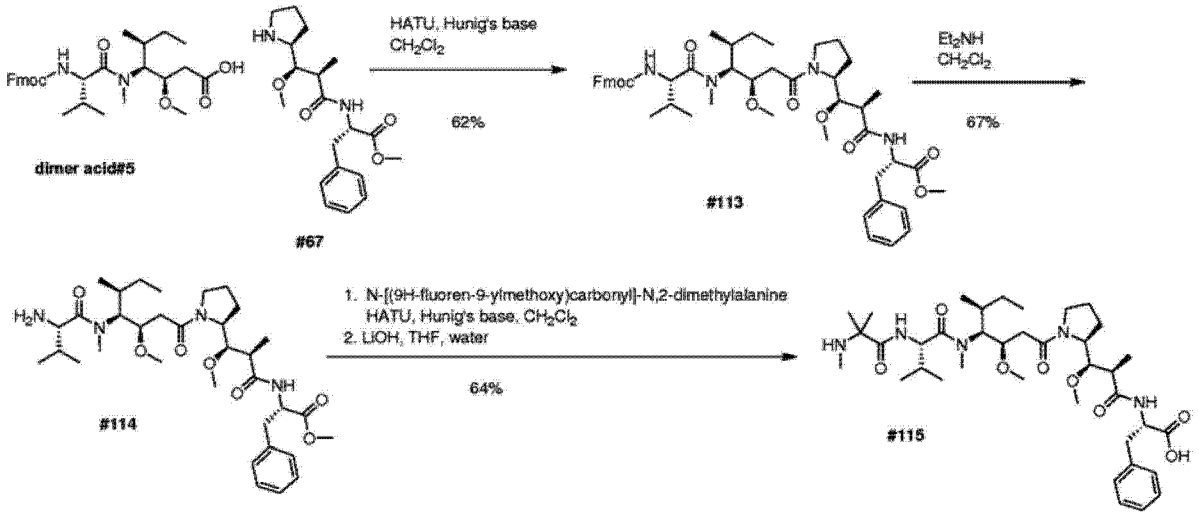
[0246] 根据一般程序 A,利用位于二氯甲烷 (10mL,0.07M) 中的 #53(701mg,0.726mmol) 合成粗制期望的材料,通过硅胶层析 (梯度 :0% 至 55% 位于庚烷中的丙酮) 纯化。用乙醚和庚烷稀释残留,并在真空中浓缩以产生白色固体 #54(406mg,75%)。LC-MS :m/z 743.6[M+H+],保留时间 = 0.70 分钟 ;HPLC(方案 A) :m/z 743.4[M+H+],保留时间 = 6.903 分钟,(纯度 >97%) ;1H NMR(400MHz,DMSO-d6),假定为旋转异构体混合物,特征信号 :δ [8.64(br d,J = 8.5Hz) 和 8.86(br d,J = 8.7Hz),总 1H],[8.04(br d,J = 9.3Hz) 和 8.08(br d,J = 9.3Hz),总 1H],[7.77(d,J = 3.3Hz) 和 7.80(d,J = 3.2Hz),总 1H],[7.63(d,J = 3.3Hz) 和 7.66(d,J = 3.2Hz),总 1H],7.13-7.31(m,5H),[5.39(ddd,J = 11,8.5,4Hz) 和 5.53(ddd,J = 12,9,4Hz), 总 1H],[4.49(dd,J = 9,8Hz) 和 4.60(dd,

J = 9,7Hz)，总 1H],3.16,3.20,3.21 和 3.25(4s，总 6H),2.93 和 3.02(2br s，总 3H),1.21(s,3H),1.13 和 1.13(2s,总 3H),[1.05(d,J = 6.7Hz) 和 1.10(d,J = 6.7Hz),总 3H],0.73-0.80(m,3H)。

[0247] 化合物 3377 的实验（示意图 #115）

[0248] N,2- 二甲基丙氨酰 -N-{(1S,2R)-4-{(2S)-2-[(1R,2R)-3-{[(1S)-1- 羰基 -2- 苯乙基] 氨基 }-1- 甲氧基 -2- 甲基 -3- 氧代丙基] 吡咯烷 -1-yl}-2- 甲氧基 -1-[(1S)-1- 甲基丙基]-4- 氧代丁基 }-N- 甲基 -L- 缬氨酰胺 - 三氟乙酸盐 (#115) 的制备。

[0249]



[0250] 步骤 1、N-{(2R,3R)-3-[(2S)-1-{(3R,4S,5S)-4-[{N-[(9H- 芴 -9-yl 甲氧基) 羰基]-L- 缬氨酰 }(甲基) 氨基]-3- 甲氧基 -5- 甲基庚酰 } 吡咯烷 -2-yl]-3- 甲氧基 -2- 甲基丙酰 }-L- 苯丙氨酰甲酯 (#113) 的合成。向氮气条件下位于 75mL 二氯甲烷中的二聚酸 #5(12.1g,23.0mM) 和 #67(11.5g,23.0mM) 的搅拌混合物中添加 HATU(10.8g,27.6mM)，随后添加 Hunig's 碱 (12.1mL,69.0mM)。在室温下搅拌反应 15 小时。反应浓缩为较小体积，用乙酸乙酯吸收并用 1N HCl 清洗 2 次。然后用盐水清洗有机层，通过硫酸钠干燥，过滤并在真空中浓缩。继而通过硅胶层析 (梯度 :0％至 70％位于庚烷中的丙酮) 纯化，产生白色固体 #113(12.3g,62％)。LC-MS(方案 Q) :m/z 855.3[M+H+],877.2[M+Na+]，保留时间 = 2.32 分钟 ;HPLC(方案 R) :/z 855.5[M+H+]，保留时间 = 9.596 分钟 (纯度 >97％)。

[0251] 步骤 2、N-{(2R,3R)-3- 甲氧基 -3-[(2S)-1-{(3R,4S,5S)-3- 甲氧基 -5- 甲基 -4-[甲基 (L- 缬氨酰) 氨基] 庚酰 } 吡咯烷 -2-yl]-2- 甲基丙酰基 }-L- 苯丙氨酰甲酯 (#114) 的合成。根据一般程序 A，利用 #113(12g,14mmol,1eq.)，二氯甲烷 (60mL,0.24M) 和二乙胺 (40mL,390mM) 合成白色 / 浅黄色固体 #114(5.9g,67％)，之后通过硅胶层析 (梯度 :0％至 25％位于二氯甲烷中的甲醇) 纯化。LC-MS(方案 Q) :m/z 633.0[M+H+]，保留时间 = 1.19 分钟。HPLC(方案 A) :/z 633.5[M+H+]，保留时间 = 7.142 分钟 (纯度 >98％)。

[0252] 步骤 3、N,2- 二甲基内氨酰 -N-{(1S,2R)-4-{(2S)-2-[(1R,2R)-3-{[(1S)-1- 羰基 -2- 苯乙基] 氨基 }-1- 甲氧基 -2- 甲基 -3- 氧代丙基] 吡咯烷 -1-yl}-2- 甲氧基 -1-[(1S)-1- 甲基丙基]-4- 氧代丁基 }-N- 甲基 -L- 缬氨酰胺 - 三氟乙酸盐 (#115) 的合成。向位于 10mL 二氯甲烷中的 N-[(9H- 芴 -9-yl 甲氧基) 羰基]-N,2- 二甲基氨基 (167mg,0.493mM)、#114(260mg,0.411mM) 和 HATU(188mg,0.493mM) 的搅拌混合物中添加

Hunig's碱(0.14mL,0.82mM)。在室温下搅拌反应1小时和20分钟。反应减少。向粗制材料中添加THF(9mL),并向该搅拌混合物中添加溶解于3mL水中的氢氧化锂(49.2mg,2.06mM)。在室温下搅拌反应4小时。反应经浓缩,继而通过中等压力反相C18层析(梯度:5%至45%于乙腈中的水,每相中含0.02% TFA)纯化白色固体#115(218mg,64%)。LC-MS(方案Q):m/z 718.7[M+H$^+$],740.6[M+Na$^+$],保留时间=1.21分钟。HPLC(方案A,45℃):m/z 718.4[M+H$^+$],保留时间=6.903分钟。

[0253] 实施例14

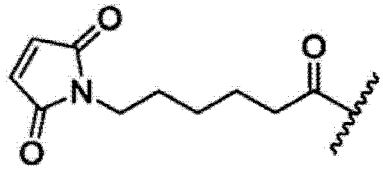
[0254] 抗IL-13-Rα2 ADC的制备

[0255] 本发明的ADC可使用具有与化合物结合的反应位点的接头部分,并导入具有与抗体的反应位点的另一接头部分而制备。在一方面,接头具有反应位点,其具有与在抗体单元例如抗体上存在的亲核基团反应的亲电子基团。抗体上的有用的亲核基团包括但不限于巯基、羟基和氨基。抗体的亲核基团的杂原子与接头上的亲电子基团反应并形成与接头的共价键。有用的亲电子基团包括但不限于马来酰亚胺和卤乙酰胺基团。

[0256] 接头具有反应位点,其具有与抗体单元上存在的亲电子基团反应的亲核基团。抗体单元上的亲电子基团提供与接头连接的便利的位点。抗体上的有用的亲电子基团包括但不限于醛基和酮基羰基。接头的亲核基团的杂原子能与抗体上的亲电子基团反应并形成与抗体的共价键。接头上的有用的亲核基团包括但不限于酰肼、肟、氨基、肼、硫代缩氨基脲、联氨羧酸酯和芳酰肼。

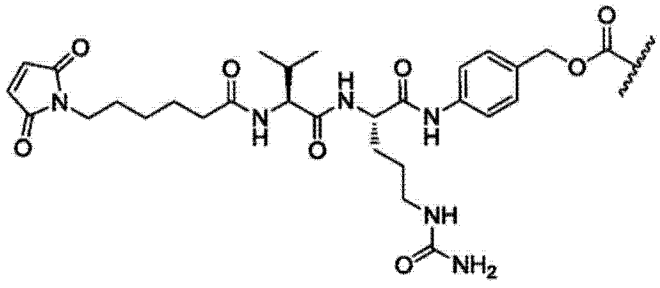
[0257] 用于本文,"mc-"指:

[0258]



[0259] 用于本文,"vc-"指:

[0260]

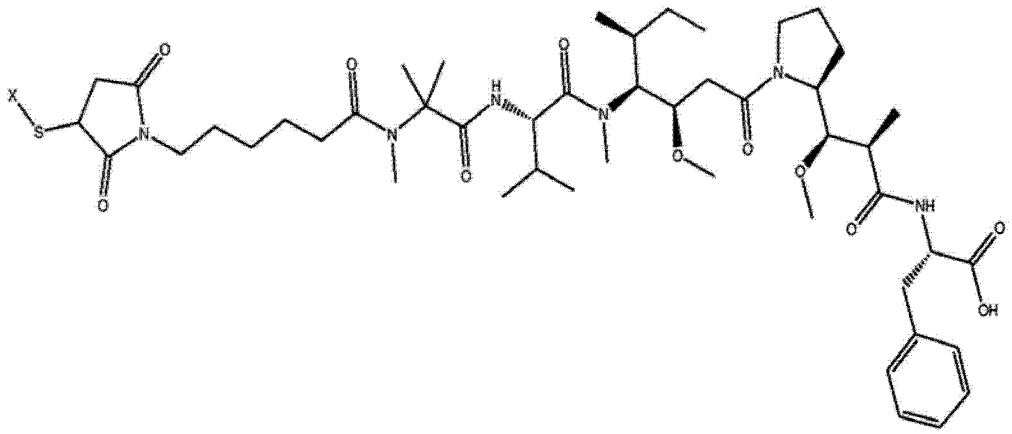


[0261] 通过用三(2-羧乙基)磷酸(TCEP)部分还原单抗随后将还原的半胱氨酸残基与期望的马来酰亚胺结尾的接头-有效负载反应制备抗IL-13-Rα2ADC。特别地,通过添加位于100mM HEPES(4-(2-羟乙基)-1-哌嗪乙磺酸缓冲液)(pH 7.0)的2.2摩尔过量的三(2-羧乙基)磷酸(TCEP)和1mM二乙烯三胺五乙酸(DTPA),于37℃经2h部分还原hu08。然而向反应混合物中添加期望的接头-有效负载,接头-有效负载/mAb摩尔比为7.0马来酰亚胺己酰基-缬氨酸-瓜氨酸-对-氨基苄氧羰基-阿里他汀-0101[vc-0101,参见以下]),或7.0马来酰亚胺己酰基-阿里他汀-3377[mc-3377,参见以下],并在15% v/v二

甲基乙酰胺 (DMA) 存在时于 25℃另外反应 1h。在 1h 孵育期后，添加 N- 己基顺丁烯二酰亚胺（对于 vc-0101 和 mc-3377 为 3 倍过量）以对未反应的巯基加帽，然后反应 15 分钟，随后添加 6 倍过量的 L-Cys 以消除任意未反应的接头 - 有效负载。反应混合物在磷酸盐缓冲盐水 (PBS)(pH 7.4) 中于 4℃透析过夜，并通过 SEC(AKTA explorer, Superdex 200 10/30 GL column) 纯化。ADC 通过尺寸排阻层析 (SEC) 表征纯度，疏水作用色谱 (HIC) 和液相色谱 - 电喷雾质谱 (LC-ESI MS) 计算药物 - 抗体比率（负载）。通过 UV 分光光度计确定蛋白浓度。

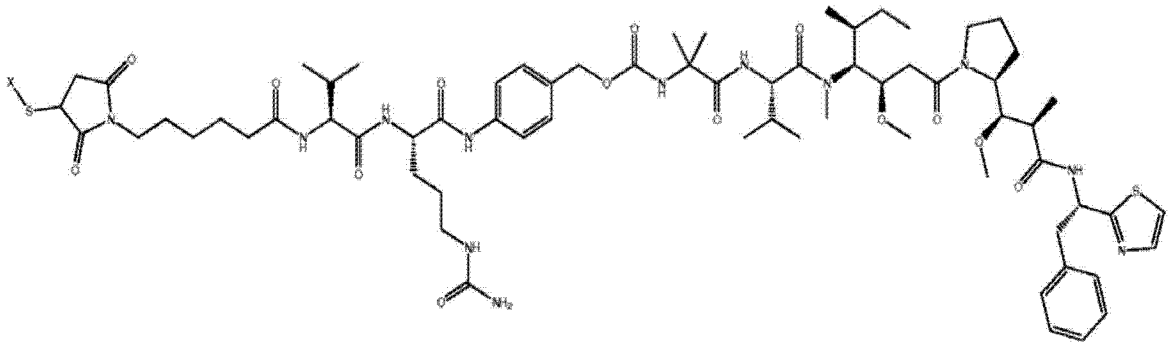
[0262] mc-3377（通过半胱氨酸残基与抗体 X 缀合）

[0263]



[0264] vc-0101（通过半胱氨酸残基与抗体 X 缀合）

[0265]



[0266] 实施例 15

[0267] 体外细胞毒性测定

[0268] 表达 IL-13-Rα2 抗原的细胞系和阴性对照细胞系与增加浓度的抗 IL-13-Rα2 ADC 培养。4 天后，测定每种培养物的活力。通过逻辑非线性回归计算 IC_{50} 值并以 ng Ab/mL 呈现。优选的接头有效负载是 vc-0101 和 mc-3377。

[0269] 人源化抗 IL-13Rα2 抗体 hu08 与表 16 中提供的各种接头 - 有效负载缀合。抗体药物缀合物根据美国专利申请 13/670,612 中记载的方法制备，其通过引用整合入本文，对应的名称和实验性化学合成示意编号如表 16 所示。

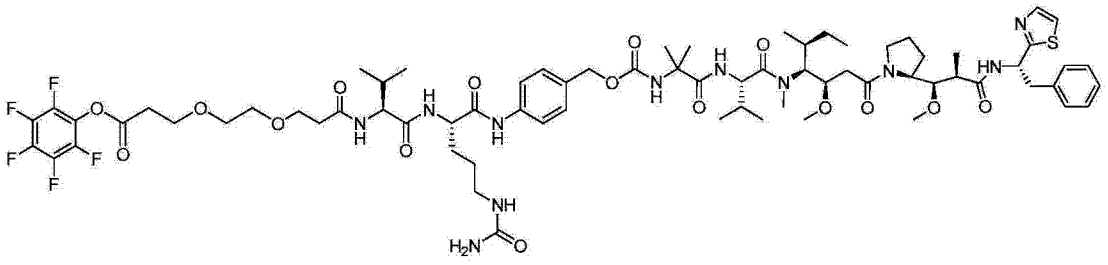
[0270] 表 16

[0271]

ADC 接头 - 有效负载	对应的 ADC 接头 - 有效负载 #
hu08-vc-0101	IL13Rα2-AB08-v1010-hG1-(C)_mcValCitPABC-#54
hu08-mc-3377	IL13Rα2-AB08-v1010-hG1-(C)_mc-#115
hu08-mc-0131	IL13Rα2-AB08-v1010-hG1-(C)_mc-0#118
hu08-Malpeg-6121	IL13Rα2-AB08-v1010-hG1-(C)_MalPeg6C2-#117
hu08-Malpeg-0131	IL13Rα2-AB08-v1010-hG1-(C)_Mal(H2O)Peg6C2-0#118
hu08-mc-6121	IL13Rα2-AB08-v1010-hG1-(C)_mc-#117
hu08-vc-3906	IL13Rα2-AB08-v1010-hG1-(C)_mcValCitPABC-#226
hu08-vc-6780	IL13Rα2-AB08-v1010-hG1-(C)_mcValCitPABC-#112
hu08-mc-8261	IL13Rα2-AB08-v1010-hG1-(C)_mc-#69
hu08-mc-3906	IL13Rα2-AB08-v1010-hG1-(C)_mc-#226
hu08-MalPeg-8261	IL13Rα2-AB08-v1010-hG1-(C)_MalPeg6C2-#69
huIgG8.8-vc-0101	huIgG8.84-mcValCitPABC-#54
huIgG8.8-mc-3377	huIgG8.84-mc-#115

[0272] 另外，如实施例 21 所述并根据标准方案制备突变版本的 hu08(hu08MAC)。化合物 0101 与可切割接头缀合以形成结构：

[0273]



[0274] 并且此后根据本文所述技术与 hu08MAC 缀合以形成 hu08MAC-0101。

[0275] 数据证实与 6 种不同的阿里他汀 (auristatin) 有效负载缀合的抗 IL-13-Rα2 抗体 hu08v1.0/1.0 有效针对测试的 IL-13-Rα2 阳性细胞系 (PC3MM2 和 A375)，其具有 1.1 至 4.9ng Ab/mL 或 7.3-32.7pM 的 IC_{50}（表 17）。另外，hu08MAC-0101 有效针对两种测试的 IL-13-Rα2 阳性细胞系 (PC3MM2 和 A375)，其具有 7.9ng Ab/mL 的 IC_{50}。所有 ADC 对 IL-13-Rα2 阴性细胞系 H460 均无活性，并且不与 IL-13-Rα2 结合的对照 ADC hIgG8.8-vc-0101 和 hIgG8.8-mc-3377 对任意测试的细胞系均无活性。

[0276] 表 17

[0277]

ADC	DAR	IC_{50} (ng Ab/mL)		
		PC3MM2	A375	H460
hu08-vc-0101	3.2	2.5	3.8	>400000
hu08-mc-3377	4.3	1.2	2.2	>400000
hu08-mc-0131	3.2	1.3	2.1	>400000
hu08-MalPeg-6121	3.3	3.5	3.4	>400000
hu08-MalPeg-0131	2.9	2.9	4.9	>400000
hu08-mc-6121	3.3	1.1	2.4	>400000
hu08-mc-3906	3	1.5	2.9	>400000
hu08 vc-6780	4	1.2	2.2	>400000
hu08MAC-0101	1.9	4.9	7.9	>400000
hIgG8.8-vc-0101	3.7	>400000	>400000	>400000
hIgG8.8-mc-3377	4.3	>400000	>400000	>400000

[0278] 实施例 16

[0279] 皮下异种移植模型

[0280] 用 PC3MM2 或 A375 肿瘤细胞皮下注射雌性无胸腺小鼠（裸鼠）。具有约 0.2 至 0.8g 晚期肿瘤的小鼠（n ＝ 8 至 10 只小鼠 / 处理组）按照 q4dx4 由静脉施用生理盐水（媒介）、具有接头 - 有效负载 vc-0101、vc-6780、vc-3906、mc-8261、mc-0131、mc-6121、mc-3377、MalPeg-8261、MalPeg-0131、MalPeg-6121 和 MalPeg-3906 的 hu08v1.0/1.0ADC，以及与 vc-0101 或 mc-3377 缀合的非结合抗体 (hIgG8.8)，剂量为 2 或 3mg 抗体 /kg。基于抗体含量确定 ADC 剂量。至少每周一次测量肿瘤，其尺寸按 mm^3 ＝ 0.5x（肿瘤宽度 ^{2}）x（肿瘤长度）计算。

[0281] 表 18 所列的体内效力结果示出不同的测试 ADC 的一系列抗肿瘤活性。潜能的相对顺序为 hu08-vc-0101>hu08-vc-6780 ≥ hu08-mc-0131>hu08-mc-6121>hu08-mc-3906>hu08-MalPeg-0131>hu08-MalPeg-6121>hu08-MalPeg-3906>hu08-mc-8261。

[0282] 表 19 中的数据表明 3mg/kg 的 ADC hu08-vc-0101 和 hu08-mc-3377 能有效降低 PC3MM2 中的肿瘤生长。与因大尺寸肿瘤（一些动物 >2500mm^3）在第 15 天终止的媒介对照组相比，第 76 天时，hu08-vc-0101 和 hu07-mc-3377 分别有 8 只中的 5 只或 8 只中的 3 只动物无可测量肿瘤。与媒介对照组类似，3mg/kg hIgG8.8-vc-0101 和 10mg/kg hIgG8.4-mc-3377 的不相关 ADC 对照组因组内的大尺寸肿瘤而分别在第 15 天和第 19 天终止。这些结果证实 hu08-vc-0101 和 hu07-mc-3377 的显著治疗效力（一些动物被治愈）是 IL-13-Rα 2 靶介导的。

[0283] 表 18

[0284]

ADC	剂量(mg/kg)Q4dx4	PC3MM2 异种移植，肿瘤体积 (mm^3 +/- SEM)							
		第-1 天	第 3 天	第 8 天 8	第 16 天	第 20 天	第 30 天	第 42 天	第 52 天
媒介	0	638±27	1149±82	1707±133	GT	GT	GT	GT	GT
hu08-Malpeg-3906	2	642±36	1036±60	1176±51	GT	GT	GT	GT	GT
hu08-mc-8261	2	642±51	1088±121	1429±158	GT	GT	GT	GT	GT
hu08-mc-0131	2	637±44	1004±73	778±83	GT	GT	GT	GT	GT
hu08-Malpeg-6121	2	638±36	947±85	1000±126	693±129	780±198	GT	GT	GT
hu08-Malpeg-0131	2	649±39	1085±54	1040±88	GT	GT	GT	GT	GT
hu08-vc-0101	2	646±36	899±54	557±49	243±28	201±20	113±17	207±49	532±151
hu08-vc-6780	2	641±28	850±100	652±54	279±55	217±45	230±133	GT	GT
hu08-mc-6121	2	636±37	909±63	821±93	441±83	414±104	GT	GT	GT
hu08-mc-3906	2	637±26	875±48	806±70	611±150	GT	GT	GT	GT
hu08-Malpeg-8261	2	645±34	991±71	1220±115	GT	GT	GT	GT	GT

[0285] GT =因大肿瘤尺寸终止组

[0286] 表 19

[0287]

ADC	剂量 (mg/kg) Q4dx4	PC3MM2 异种移植，肿瘤体积(mm^3+/-SEM)							
		第-1 天	第 2 天	第 10 天	第 19 天	第 40 天	第 51 天	第 61 天	第 71 天
媒介	0	340 ± 16	573 ± 47	1441 ± 176	1975 ± 272	GT	GT	GT	GT
hIgG8.84-mc-3377	10	328 ± 27	459 ± 63	295 ± 121	544 ± 258	GT	GT	GT	GT
hu08-mc-3377	3	337 ± 21	385 ± 36	41 ± 12	0 ± 0	78 ± 36	346 ± 147	616 ± 243	902 9 364
hu08-vc-0101	3	339 ± 18	433 ± 45	38 ± 14	6 ± 6	110 ± 110	230 ± 230	GT	GT
hIgG8.8-vc-0101	3	333 ± 23	449 ± 15	686 ± 134	GT	GT	GT	GT	GT

[0288] GT =因大肿瘤尺寸终止组

[0289] 表 20a 中的数据表明 hu08-vc-0101 和 hu08-mc-3377 的 ADC 在 3mg/kg 时在使用 A375 细胞的第二个体内异种移植模型中有效。媒介对照组和 hIgG8.8-vc-0101 与 hIgG8.8-mc-3377 的不相关 ADC 对照组因大肿瘤尺寸而分别在第 19、22、27 天终止。用 3mg/kg 的 hu08-vc-0101 和 hu08-mc-3377 处理造成所有动物中的肿瘤消退并提供显著的存活优势。用 hu08-mc-3377 处理第 19-22 天的所有动物中均未观察到可测量的肿瘤。尽管一些肿瘤复发，但在第 71 天有 10 只动物中的 5 只没有可测量的肿瘤。用 hu08-vc-0101 处理组检测 100 天，在第 19-100 天有 8 只动物没有可测量的肿瘤。这些结果证实 hu08-vc-0101

和 hu08-mc-3377 对 IL-13-Rα2 阳性肿瘤强的抗肿瘤活性。

[0290] 表 20a

[0291]

ADC	剂量 (mg/kg) Q4dx4	A375 异种移植肿瘤体积 (mm^3 ± SEM)							
		第-1 天	第 2 天	第 13 天	第 19 天	第 40 天	第 51 天	第 61 天	第 71 天
媒介	0	340 ± 16	573 ± 47	1441 ± 176	1975 ± 272	GT	GT	GT	GT
hIgG8.8-mc-3377	3	328 ± 27	459 ± 63	295 ± 121	544 ± 258	GT	GT	GT	GT
Hu08-mc-3377	3	337 ± 21	385 ± 36	41 ± 14	0 ± 0	78 ± 36	346 ± 147	616 ± 243	902 ± 364
Hu08-vc-0101	3	336 ± 19	407 ± 42	43 ± 15	7 ± 15	122 ± 7	256 ± 122	0 ± 0	0 ± 0
hIgG8.8-vc-0101	3	333 ± 23	449 ± 15	686 ± 135	1145 ± 212	GT	GT	GT	GT

[0292] GT ＝因大肿瘤尺寸终止组

[0293] 表 20b 和 20c 中的数据表明 ADC hu08-vc-0101 和 hu08-mc3377 在三分之一的使用 HEY-C2 卵巢癌细胞系体内异种移植模型中有效。媒介对照组和阴性 ADC 对照组的 hIgG8.8-vc0101(3mg/kg 和 10mg/kg) 及 hIgG8.8-mc-3377(10mg/kg) 因大肿瘤尺寸终止，标示为 GT。以 1、3 和 10mg/kg 剂量水平的 hu08-vc-0101 和 hu08-mc-3377 处理提供剂量依赖性应答。用 3mg/kg hu08-vc-0101 处理的 9 只动物中的 5 只和用 10mg/kg hu08-vc-0101 处理的 9 只动物中的 7 只存活至研究结束（第 103 天）。相似地，用 3mg/kg hu08-vc-3377 处理的 9 只动物中的 5 只和用 10mg/kg hu08-vc-3377 处理的 9 只动物中的 9 只存活至研究结束（第 103 天）。

[0294] 表 20b

[0295]

ADC	剂量 (mpk) Q4d	HEY-C2 异种移植，肿瘤体积 (mm^3± SEM)											
		第 -1 天	第 3 天	第 12 天	第 23 天	第 30 天	第 40 天	第 51 天	第 60 天	第 72 天	第 79 天	第 95 天	第 103 天
媒介	0	211 ± 16	375 ± 21	919 ± 53	2311 ± 156	GT	GT	GT	GT	GT	GT	GT	GT
hAB08 mc_3377	1	211 ± 17	324 ± 23	337 ± 25	510 ± 128	665 ± 203	GT	GT	GT	GT	GT	GT	GT
	3	211 ± 17	319 ± 38	250 ± 37	207 ± 41	239 ± 64	336 ± 131	602 ± 258	GT	GT	GT	GT	GT
	10	211 ± 16	303 ± 28	181 ± 17	114 ± 10	106 ± 7	84 ± 16	69 ± 14	53 ± 14	42 ± 11	41 ± 12	121 ± 59	308 ± 166
hIgG 8.8 mc_3377	10	212 ± 18	375 ± 46	557 ± 53	650 ± 183	GT	GT	GT	GT	GT	GT	GT	GT

[0296] 表 20c

[0297]

ADC	剂量 (mpk) Q4d	HEY-C2 异种移植，肿瘤体积 (mm^3± SEM)											
		第 -1 天	第 3 天	第 12 天	第 23 天	第 30 天	第 40 天	第 51 天	第 60 天	第 72 天	第 79 天	第 95 天	第 103 天
hAB08 vc_0101	1	211 ± 16	335 ± 27	331 ± 43	479 ± 96	697 ± 177	GT	GT	GT	GT	GT	GT	GT
	3	210 ± 18	324 ± 19	258 ± 36	200 ± 56	222 ± 90	354 ± 239	GT	GT	GT	GT	GT	GT
	10	211 ± 15	333 ± 16	295 ± 29	204 ± 26	142 ± 15	94 ± 14	66 ± 13	83 ± 35	208 ± 129	324 ± 219	GT	GT
hIgG 8.8 vc_0101	3	212 ± 22	384 ± 35	567 ± 95	963 ± 204	GT	GT	GT	GT	GT	GT	GT	GT
	10	211 ± 20	354 ± 37	349 ± 48	198 ± 38	151 ± 31	165 ± 33	413 ± 128	922 ± 287	GT	GT	GT	GT

[0298] GT ＝因大肿瘤尺寸终止组

[0299] 实施例 17

[0300] 半胱氨酸突变产生位点特异性缀合

[0301] 进行接头-有效负载与抗体的位点特异性缀合，以改善同种药物负载并避免具有改变的抗原结合或改变的药物动力学的ADC亚群，这些常见于传统缀合方法。一种该位点特异性缀合方法是在靶抗体氨基酸序列中的特定位点引入半胱氨酸残基。之前已经鉴定出重链恒定区和轻链恒定区中的一些氨基酸位置（参见美国专利申请61/580,169）并在hu08v1.0/1.0抗体的特定氨基酸位置被半胱氨酸残基取代。所有半胱氨酸突变均基于pSMED-hu08v1.0和pSEMN3-hu08v1.0通过定点诱变或重叠PCR构建。以下是源自hu08v1.0/1.0的半胱氨酸突变体列表及对应的SEQ ID号（表21）。

[0302] 表21

[0303]

抗体		突变位点	氨基酸 SEQ ID NO:
单突变体	HC	L443C	28
		Q347C	29
	LC	kA111C	30
		kK183C	31
		kK188C	32
双突变体	HC	L443C/K392C	33
		L443C/V422C	34
	HC/LC	L443C/kA111C	28/30
		L443C/kK183C	28/31
		Q347C/kA111C	29/30
		Q347C/kK183C	29/31

[0304] 实施例18

[0305] 半胱氨酸突变体的表征

[0306] 在自由293表达培养基（Invitrogen,calsdad,CA）中的瞬时转染的HEK293悬浮细胞培养物或CHO细胞培养物稳定株中表达hu08v1.0/1.0的半胱氨酸突变体。在标准条件下通过蛋白A(ProA)层析从细胞培养基中分离抗体。使用配备30,000MWCO膜的Millipore旋转管对柱级分进行富集和浓缩。然后将蛋白上样于用pH 7.2的PBS-CMF平衡的尺寸排阻柱（Superdex200）。使用配备30,000MWCO膜的Millipore旋转管对峰值级分进行富集和浓缩，并最终通过0.22um过滤器进行过滤。来自瞬时表达的数据示于表22，来自CHO细胞稳定株的数据示于表23。野生型hu08v1.0/1.0及其半胱氨酸衍生物在ProA捕获后具有类似的终产量和纯度，SEC后纯度达99％，并在2-3轮冷冻和融化后稳定。该数据证实与hu08v1.0/1.0相比，半胱氨酸突变体保留其表达属性和纯化性能。

[0307] 表22

[0308]

抗体名称	终产量（mg/L）	纯度（ProA捕获）	纯度（SEC）	F/T 2-3轮
hu08v1.0/1.0	26.5	97％	ND	稳定

hu08v1.0/1.0-L443C	56.0	94%	99%	稳定
hu08v1.0/1.0-Q347C	30.0	97%	99%	稳定
hu08v1.0/1.0-A111C	52.8	96.4%	99%	稳定
hu08v1.0/1.0-K183C	44.4	97.0%	99%	稳定
hu08v1.0/1.0-K188C	27.9	96.2%	99%	稳定
hu08v1.0/1.0-K392C/L443C	28.0	85.0%	99%	稳定
hu08v1.0/1.0-V422C/L443C	40.5	99.7%	ND	稳定
hu08v1.0/1.0-Q347C/A111C	31.4	96.6%	99%	稳定
hu08v1.0/1.0-L443C/A111C	43.7	ND	99%	稳定
hu08v1.0/1.0-Q347C/K183C	29.0	97.2%	99%	稳定
hu08v1.0/1.0-L443C/K183C	57.0	ND	99%	稳定

[0309] 表 23

[0310]

抗体名称	终产量 (mg/L)	纯度 (ProA 捕获)	纯度 (SEC)	F/T 2-3 轮
hu08v1.0/1.0-Q347C	26.74	94.0%	99.0%	稳定
hu08v1.0/1.0-K392C/L443C	38.33	89.0%	99.0%	稳定
hu08v1.0/1.0	91.0	95%	99.0%	稳定

[0311] hu08v1.0/1.0 半胱氨酸突变体的结合性能

[0312] 通过标准 ELISA 和竞争 ELISA 评价半胱氨酸突变体与 hIL-13-Rα2 的结合性能。结果表明,所有的半胱氨酸突变体均具有与野生型 hu08v1.0/1.0 相似的 ED50(表 23)。该结论得到使用生物素化 ch08 的竞争 ELISA 的证实。表 24 证实,所有的半胱氨酸突变体均具有与野生型 hu08 相似的 IC50,表明结合亲和力与野生型 hu08 相同。ch07 用作阴性对照。

[0313] 表 24

[0314]

	ED50(nM)	IC50(nM)
hu08	0.11	2.01
hu08/L443C	0.10	2.25

hu08/L443C/K392C	0.09	2.10
hu08/L443C/V422C	0.09	2.17
hu08/L443C/kA111C	0.12	2.32
hu08/L443C/kK183C	0.11	2.45
hu08/Q347C	0.10	2.07
hu08/Q347C/kA111C	0.10	2.44
hu08/Q347C/kK183C	0.13	2.41
hu08/kA111C	0.22	4.07
hu08/kK183C	0.11	2.47
hu08/kK188C	0.18	2.73
hu08/kD185A	0.108	2.364
ch07	0.10	2.11

[0315] hu08v1.0/1.0 半胱氨酸突变体的热稳定性

[0316] 使用 MicroCal 的装配自动进样器的 Capillary-DSC 系统 (Northampton,MA),通过毛细管差示扫描量热法 (DSC) 对抗 IL-13-Rα2 半胱氨酸突变体单抗的热稳定性进行分析。使用标准方案。记录样品细胞和参考细胞之间的热容量差,并在 Origin7.0 软件 (OriginLab,Northampton,MA) 中使用与 3 个热跃迁适应的非 -2 状态模型 (non-2 states model fit to 3 thermal transitions) 进行分析。并在样品和参考细胞中使用 PBS 缓冲液产生基线热图,用于减去与蛋白变性无关的任何系统热。表 25 中的结果显示 2 种单半胱氨酸突变体抗体和 3 种双半胱氨酸突变体抗体具有与母系 hu08v1.0/1.0 类似的 Tm。

[0317] 表 25

[0318]

hu08v1.0/1.0 及其半胱氨酸突变体		DSC (差示扫描量热法)		
		Tm1 (℃) CH2	**Tm2 (℃)Fab**	**Tm3 (℃) CH3**
单突变体	L443C	72.85±0.17	80.30±0.02	
	Q347C	72.78±0.15	80.18±0.02	
双突变体	K392C/L443C	74.09±0.27	80.17±0.22	78.14±0.15
	Q347C/kK183C	72.59±0.23	79.82±0.12	
	L443C/kK183C	72.68±0.12	79.93±0.02	85.58±0.18
hu08v1.0/1.0		73.48±0.19	80.29±0.02	85.48±0.14

[0319] hu08v1.0/1.0 半胱氨酸突变体 ADC 的热稳定性

[0320] 野生型 hu08v1.0/1.0 和 5 种半胱氨酸突变体如美国临时专利申请 61/580,169 所述与 vc-0101 缀合。通过差示扫描量热法 (DSC,详见上述) 测量所有 ADC 的热稳定性。如下表 26 所示,野生型 hu08v1.0/1.0 vc-0101 缀合物的 Tm1 显著低于 (≥ 5℃) 其裸抗体 (参见表 8)。ADC 的 Tm2 比裸抗体低约 1-2℃,而 Tm3 类似。对于 hu08v1.0/1.0 半胱氨酸突变体,vc-0101 缀合物的 Tm1、Tm2 和 Tm3 与其对应裸抗体类似或略低 (≤ 1-2℃)(参见表 25)。这表明缀合后半胱氨酸突变体比野生型抗体更加热稳定。

[0321] 表 26

[0322]

抗体		缀合		DSC (差示扫描量热法)			
		接头有效负载	DAR	Tm1 (℃) CH2	Tm2 (℃) Fab		Tm3 (℃) CH3
单突变	L443C	vc-0101	2.1	73.27±0.19	79.29±0.01		
	Q347C	vc-0101	2.1	71.49±0.09	79.16±0.59		77.25±0.59
双突变	K392C/L443C	vc-0101	3.7	69.02±0.07	78.83±0.02		76.70±0.11
	Q347C/kK183C	vc-0101	4.3	70.15±0.08	78.21±0.12		76.20±1.10
	L443C/kK183C	vc-0101	4.0	71.30±0.13	77.85±0.02		84.16±0.11
hu08v1.0/1.0		vc-0101	3.2	68.42±0.09	77.77±0.10	79.45±0.02	85.03±0.07

[0323] 与 vc-0101 缀合的 hu08v1.0/1.0 半胱氨酸突变体的血浆和谷胱甘肽稳定性

[0324] 用于谷胱甘肽稳定性的样品制备 :30μg 于 PBS 中的 hu08-vc-0101 ADC 或 hu08- 半胱氨酸突变体 -vc-0101 ADC 与谷胱甘肽 (GSH) 溶液混合,产生终浓度 0.5mM 的 GSH。0.5mM GSH 中的 ADC 和对照 ADC(0mM GSH) 在 37℃孵育并在第 0、3 和 6 天采样。TCEP(三 (2- 羧乙基) 磷酸) 用于还原。

[0325] 用于小鼠血浆稳定性的样品制备 :90μg 于 PBS 中的 ADC 样品与小鼠血浆混合,用 20% MPER(哺乳动物蛋白提取试剂)1:1 稀释。ADC/ 血浆样品于 37℃孵育并在第 0、1 和 2 天取等分并用生物素化的重组 hIL-13-Rα 蛋白免疫沉淀。用 0.15% 甲酸溶液洗脱 ADC,并用浓缩的 Tris HCl 缓冲液中和至 pH 7.8。样品通过添加 PNGaseF(肽 :N- 糖苷酶 F) 去糖基化并用 TCEP 还原。

[0326] LC/MS 分析过程 :通过添加含 10% 乙腈的 0.1% 甲酸溶液对 ADC/ 血浆和 ADC/GSH 稳定性样品等分进行酸化,然后在与 Water Xevo G2 Q-TOF 质谱仪偶联的 Agilent 1100 capillary HPLC 上进行 LC/MS 分析。用 0.1% 甲酸溶液将分析物上样于 Zorbax Poroshell 300SB C8 柱 (0.5mm×75mm,保持在 80℃),并用 20 - 40% 缓冲液 B(80% 乙腈,18% 1- 丙醇,2% 水,0.1% 甲酸) 的梯度以 20μl/min 流速经 5.5 分钟洗脱。以设置为 3.3kV 的毛细管电压通过阳性、灵敏度模式进行质谱检测。用 MassLynx 中的 MaxEnt 1 函数进行数据分析,基于以下公式,强度用作荷载计算 :

[0327] 荷载 = 2*[LC1/(LC0+LC1)]+2*HC1/(HC0+HC1+HC2)]+4*HC2/(HC0+HC1+HC2)。

[0328] hu08 及其与 vc-0101 缀合的半胱氨酸突变体的 ADC(来自表 26) 接受血浆和 GSH 稳定性测定。结果证实,来自半胱氨酸突变体的 ADC 比来自传统缀合技术的 ADC 更稳定 (表 27)。

[0329] 表 27

[0330]

半胱氨酸突变体-vc-0101 ADC		血浆稳定性	GSH 稳定性
		荷载% 第 2 天	荷载% 第 6 天
单突变体	L443C	95.0%	94.7%
	Q347C	95.0%	100.0%
双突变体	K392C/L443C	89.7%	100.0%
	Q347C/kK183C	81.6%	97.4%
	L443C/kK183C	91.9%	86.8%
hu08		___	62.5%

[0331] 实施例 19

[0332] 半胱氨酸突变体 ADC 的体外细胞毒性测定

[0333] 用增加浓度的与 vc-0101 或 mc-3377 缀合的 hu08v1.0/1.0 半胱氨酸突变体培养表达 IL-13-Rα2 抗原的细胞系或 IL-13-Rα2 阴性细胞系 H460。4 天后，评定每种培养物的生存力。通过逻辑非线性回归计算 IC_{50}值并以 ng/mL 呈现（表 28a 和 28b）。

[0334] 表 28a

[0335]

*A*DC (变体-vc-0101)	DAR	IC_{50} (ng/mL)	
		PC3MM2	A375
野生型 hu08	3.2	1.93±0.22	1.70±0.98
Q347C/kK183C	4.3	1.77±0.46	1.13±0.15
Q347C	2.1	2.66±0.60	1.65±0.09
L443C	2.1	2.40	1.41
K392C/L443C	3.7	1.41	0.63
L443C/kK183C	4.0	1.72	0.83

[0336] 表 28b

[0337]

ADC (变体-mc-3377)	DAR	IC_{50} (ng Ab/mL)		
		PC3MM2	A375	H460
野生型 hu08	3.5	1.8	1.8	>400000
L443C	2.3	3.9	3.3	>400000
K392C+L443C	3.5	2.2	1.3	>400000
L443C+kK183C	4	1.9	2.3	>400000

[0338] 实施例 20

[0339] 半胱氨酸突变体 ADC 的皮下异种移植模型

[0340] 用 PC3MM2 肿瘤细胞皮下注射雌性无胸腺小鼠（裸鼠）。具有约 0.2 至 0.5g 晚期肿瘤的小鼠（n ＝ 8 至 10 只小鼠 / 处理组）以 1.5 或 4.5mg/kg 的单剂量由静脉施用生理盐水（媒介）、半胱氨酸突变体 L443C-vc-0101、K392C/L443C-vc-0101、L443C/K183C-vc-0101、Q347C-vc-0101 或 hAB-vc-0101，或 3、6 和 12mg/kg 的 L443C-mc-3377、K392C/L443C-mc-3377、L443C/K183C-mc-3377。基于抗体含量确定所有 ADC 剂量。至少每周一次测量肿瘤，其尺寸（mm^3 ± SEM）按 mm^3 ＝ 0.5x（肿瘤宽度 ^{2}）x（肿瘤长度）计算。

[0341] 表 29 中数据表明，与媒介对照组相比，1.5mg/kg 和 4.5mg/kg 的 L443C-vc-0101、K392C/L443C-vc-0101、L443C/K183C-vc-0101 和 hu08-vc-0101 均抑制 PC3MM2 异种移植的生长。L443C/K183-vc-0101 是实验中所测试的最强力化合物，其 4.5mg/kg 的剂量水平具有在组终止前最长 70 天的监测时间。

[0342] 表 29

[0343]

ADC	剂量 单剂量	PC3MM2 异种移植，肿瘤体积(mm^3±SEM)											
		第 0 天	第 3 天	第 6 天	第 10 天	第 13 天	第 18 天	第 21 天	第 32 天	第 42 天	第 53 天	第 63 天	第 70 天
媒介		341 ± 8	469 ± 25	681 ± 42	857 ± 60	1058 ± 115	1272 ± 167	GT	GT	GT	GT	GT	GT
L443C vc-0101	1.5	353 ± 26	421 ± 51	429 ± 65	428 ± 65	472 ± 93	567 ± 133	629 ± 176	GT	GT	GT	GT	GT
K392C+L443C vc-0101	1.5	343 ± 10	441 ± 31	417 ± 22	344 ± 23	356 ± 27	479 ± 57	565 ± 61	1219 ± 154	1411 ± 157	GT	GT	GT
L443C+Kk183C vc-0101	1.5	350 ± 18	431 ± 35	453 ± 49	396 ± 44	390 ± 49	464 ± 46	446 ± 54	842 ± 171	GT	GT	GT	GT
hu08-vc-0101	1.5	335 ± 16	451 ± 28	468 ± 47	439 ± 65	461 ± 86	600 ± 116	680 ± 135	1502 ± 406	GT	GT	GT	GT
L443C-vc-0101	4.5	358 ± 8	397 ± 13	334 ± 24	199 ± 25	164 ± 17	143 ± 23	124 ± 20	156 ± 47	242 ± 86	509 ± 193	618 ± 232	GT
K392C+L443C- vc-0101	4.5	351 ± 19	363 ± 27	353 ± 28	196 ± 10	169 ± 9	136 ± 17	118 ± 27	230 ± 98	318 ± 142	553 ± 235	GT	GT
L443C+Kk183C-vc-0101	4.5	343 ± 17	417 ± 32	397 ± 44	219 ± 18	156 ± 18	121 ± 8	93 ± 10	105 ± 27	175 ± 77	461 ± 221	423 ± 134	694 ± 222

[0344] GT ＝因大肿瘤尺寸终止组

[0345] 表 30 中的数据表明，与媒介对照组相比，1.5mg/kg 和 4.5mg/kg 的 Q347C-vc-0101 和 Q347C/K183C-vc-0101 均抑制 PC3MM2 异种移植的生长。另外，数据证实与媒介对照组相比，1.5mg/kg 的 hu08MAC-0101 抑制 PC3MM2 异种移植的生长。Q347C/K183C-vc-0101 是实验中所测试的最强力化合物，其 4.5mg/kg 的剂量水平具有最长 77 天的监测时间。这些结果表明，位点特异性 vc-0101 缀合物与野生型 hu08-vc-0101 缀合物效力类似或更优。

[0346] 表 30

[0347]

ADC	剂量	PC3MM2 异种移植，肿瘤体积(mm^3±SEM)									
	单剂量	第 0 天	第 5 天	第 8 天	第 12 天	第 15 天	第 20 天	第 30 天	第 41 天	第 55 天	第 77 天
媒介	0	325 ± 9	590 ± 41	782 ± 79	1140 ± 142	GT	GT	GT	GT	GT	GT
Q347+kC183-vc-0101	1.5	337 ± 17	383 ± 39	324 ± 35	347 ± 46	381 ± 63	481 ± 89	770 ± 121	GT	GT	GT
Q347-vc-0101	1.5	333 ± 11	341 ± 14	308 ± 32	309 ± 39	352 ± 54	473 ± 79	757 ± 238	GT	GT	GT
hu08MAC-0101	1.5	328 ± 49	393 ± 96	352 ± 106	432 ± 124	556 ± 236	732 ± 305	GT	GT	GT	GT
Q347+kC183-vc-0101	4.5	336 ± 17	252 ± 19	171 ± 18	145 ± 13	128 ± 8	88 ± 14	113 ± 37	28 ± 28	75 ± 75	128 ± 128
Q347-vc-0101	4.5	338 ± 16	278 ± 28	176 ± 20	136 ± 16	130 ± 30	128 ± 41	267 ± 112	GT	GT	GT
hu08-vc-0101	1.5	333 ± 12	431 ± 40	281 ± 25	299 ± 32	362 ± 47	450 ± 58	956 ± 166	GT	GT	GT

[0348] GT ＝因大肿瘤尺寸终止组

[0349] 表 31a 和 b 中的数据表明，与媒介对照组相比，L443C-mc-3377、K392C/L443C-mc-3377、L443C/K183C-mc-3377 和 hu08-mc-3377 以剂量依赖方式造成肿瘤退化。L443C/K183C-mc-3377 是实验中所测试的最强力化合物，与其他化合物相比，其 12mg/kg 的剂量水平具有小的平均肿瘤尺寸。另外，用 12mg/kg 的 L443C/K183C-mc3377 处理组的 8 只动物中有 6 只在第 60 天时无可测量肿瘤。

[0350] 表 31a

[0351]

ADC	剂量 (mg/kg) 单剂量	PC3MM2 异种移植，肿瘤体积 (mm^3 +/- SEM)								
		第 0 天	第 7 天	第 14 天	第 21 天	第 28 天	第 38 天	第 45 天	第 50 天	第 60 天
媒介	0	339 ± 19	1021 ± 112	1737 ± 194	GT	GT	GT	GT	GT	GT
C443 mc_3377	3	341 ± 24	350 ± 61	461 ± 82	601 ± 139	954 ± 250	GT	GT	GT	GT
	6	333 ± 31	352 ± 51	280 ± 54	366 ± 103	501 ± 147	1030 ± 307	GT	GT	GT
	12	338 ± 27	293 ± 45	177 ± 39	153 ± 22	278 ± 52	559 ± 117	752 ± 179	871 ± 267	GT
C392+C443 mc_3377	3	341 ± 26	387 ± 64	462 ± 132	496 ± 197	977 ± 381	GT	GT	GT	GT
	6	335 ± 31	237 ± 37	123 ± 37	133 ± 48	179 ± 85	469 ± 193	606 ± 252	768 ± 334	GT
	12	340 ± 23	312 ± 40	213 ± 45	150 ± 49	208 ± 79	261 ± 94	507 ± 242	695 ± 319	GT
C443+kC183 mc_3377	3	343 ± 27	386 ± 50	336 ± 60	452 ± 125	809 ± 245	GT	GT	GT	GT
	6	332 ± 28	311 ± 58	189 ± 32	135 ± 27	129 ± 37	237 ± 101	385 ± 185	491 ± 271	GT
	12	336 ± 32	238 ± 44	103 ± 30	49 ± 25	65 ± 26	61 ± 52	70 ± 55	109 ± 87	158 ± 123

[0352] 表 31b

[0353]

ADC	Dose (mg/kg) single dose	PC3MM2 异种移植，肿瘤体积(mm^3 +/- SEM)								
		第 0 天	第 7 天	第 14 天	第 21 天	第 28 天	第 38 天	第 45 天	第 50 天	第 60 天
mc_3377	3	336 ± 29	340 ± 33	285 ± 75	436 ± 131	732 ± 265	GT	GT	GT	GT
	6	327 ± 35	231 ± 45	89 ± 29	30 ± 16	84 ± 25	184 ± 42	285 ± 108	402 ± 158	GT
	12	340 ± 28	180 ± 14	81 ± 28	52 ± 28	76 ± 45	171 ± 102	282 ± 179	354 ± 233	442 ± 328

[0354] GT ＝因大肿瘤尺寸终止组

[0355] 实施例 21

[0356] 使用 MAC 技术的位点特异性缀合

[0357] 制备包含抗体或其抗原结合片段的多功能抗体缀合物 (MAC) 的方法之前已有记载 (WO2012/007896 和美国专利 61/584,675)。本发明的一个方面是使用抗体或其抗原结合片段制备特异性结合人 IL-13Rα2 的 MAC，其中所述抗体在 SEQ ID NO:52 所示的 LC 的第 185 位具有突变 D185A，并且该抗体通过与 SEQ ID NO:49 所示的 LC 的 K188 侧链结合的接头与至少一种药物部分共价缀合；所述方法包含：使用 PFP（五氟苯酚）酯共价连接药物部分，并使形成的效应物部分－接头－离去基团复合物与抗体在约 3.5:1 至约 4.5:1 药物部分：抗体的摩尔比反应。在一些方面，所述摩尔比是约 3.7:1 至约 4.3:1。本文所述 MAC 命名为 hu08MAC-0101 并包含 SEQ ID NO:52 所示的 LC 的突变 D185A；以及药物部分 0101（实施例 13），其与 SEQ ID NO:52 所示的 LC 的第 188 位置处的赖氨酸残基 (K188) 的侧链缀合。hu08MAC-0101 的体外活性示于表 17（实施例 15）。PC3MM2 异种移植模型的体内活性示于表 30（实施例 20）。

[0001]

序列表

<110> 辉瑞公司

<120> 抗 IL-13 受体 α 2 抗体和抗体-药物缀合物

<130> PC71968PRV4

<160> 55

<170> PatentIn version 3.5

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<211> 122
<212> PRT
<213> Artificial Sequence

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<223> Synthetic Peptide Sequence

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Asn
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Thr Val Ser Ser Gly Gly Ser Tyr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gln Gly Thr Thr Ala Leu Ala Thr Arg Phe Phe Asp Val Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 2
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<212> PRT
<213> Artificial Sequence

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<223> Synthetic peptide sequence

<400> 2

Ser Arg Asn Gly Met Ser
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<210> 3
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 3

[0002]

Thr Val Ser Ser Gly Gly Ser Tyr Ile Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 4
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

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Gln Gly Thr Thr Ala Leu Ala Thr Arg Phe Phe Asp Val
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<210> 5
<211> 107
<212> PRT
<213> Artificial Sequence

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<223> Synthetic peptide sequence

<400> 5

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Val Gly Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Tyr Arg Ser Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His His Tyr Ser Ala Pro Trp
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 6
<211> 11
<212> PRT
<213> Artificial Sequence

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<223> Synthetic peptide sequence

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Lys Ala Ser Gln Asp Val Gly Thr Ala Val Ala
1 5 10

<210> 7
<211> 7

[0003]

<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 7

Ser Ala Ser Tyr Arg Ser Thr
1 5

<210> 8
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 8

Gln His His Tyr Ser Ala Pro Trp Thr
1 5

<210> 9
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 9

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Thr Lys Tyr
20 25 30

Gly Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Lys Trp Ala Gly Gly Ser Thr Asp Tyr Asn Ser Ala Leu Met
50 55 60

Ser Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Asp His Arg Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser
115

<210> 10
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 10

[0004]

Thr Lys Tyr Gly Val His
1 5

<210> 11
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 11

Val Lys Trp Ala Gly Gly Ser Thr Asp Tyr Asn Ser Ala Leu Met Ser
1 5 10 15

<210> 12
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

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Asp His Arg Asp Ala Met Asp Tyr
1 5

<210> 13
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 13

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Thr Ala Ser Leu Ser Val Ser Ser Thr
20 25 30

Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln
65 70 75 80

Pro Glu Asp Phe Ala Thr Tyr Tyr Cys His Gln Tyr His Arg Ser Pro
85 90 95

Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

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<211> 12
<212> PRT
<213> Artificial Sequence

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[0005]

<223> Synthetic peptide sequence

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Thr Ala Ser Leu Ser Val Ser Ser Thr Tyr Leu His
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<210> 15
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 15

Ser Thr Ser Asn Leu Ala Ser
1 5

<210> 16
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 16

His Gln Tyr His Arg Ser Pro Leu Thr
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<210> 17
<211> 366
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic nucleotide sequence

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tcttgtgccg cctccggctt caccttcagt aggaatggca tgtcttgggt gaggcaggcc 120

cctggcaagg gcctggagtg ggtggccacc gttagtagtg gtggtagtta catctactat 180

gcagacagtg tgaaggggcg gttcaccatc tccagggaca acgccaagaa ctccctgtac 240

ctccagatga actccctgag ggccgaggat accgccgtgt actactgtgc cagacaaggg 300

actacggcac tagctacgag gttcttcgat gtctggggcc agggcaccct ggtgaccgtg 360

tcctct 366

<210> 18
<211> 321
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic nucleotide sequence

<400> 18
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atcacctgta aggccagtca ggatgtaggt actgctgtag cctggtatca gcagaagcct 120

ggcaaggctc ccaagctgct gatctactcg gcatcctacc ggtccactgg cgtgccttcc 180

agattctccg gctctggctc tggcaccgat ttcaccctga ccatctcctc cctccagcct 240

[0006]

gaggatttcg ccacctacta ctgccagcac cattatagtg ctccgtggac gtttggcggc 300

ggaacaaagg tggagatcaa g 321

<210> 19
<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 19

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Asn
20 25 30

Gly Met Ser Trp Val Arg Gln Thr Pro Asp Lys Arg Leu Glu Trp Val
35 40 45

Ala Thr Val Ser Ser Gly Gly Ser Tyr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gln Gly Thr Thr Ala Leu Ala Thr Arg Phe Phe Asp Val Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 20
<211> 366
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic nucleotide sequence

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cctgacaagc gcctggagtg ggtggccacc gttagtagtg gtggtagtta catctactat 180

gcagacagtg tgaaggggcg gttcaccatc tccagggaca acgccaagaa ctccctgtac 240

ctccagatga gctccctgag ggccgaggat accgccgtgt actactgtgc cagacaaggg 300

actacggcac tagctacgag gttcttcgat gtctggggcc agggcaccct ggtgaccgtg 360

tcctct 366

<210> 21
<211> 107
<212> PRT
<213> Artificial Sequence

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[0007]

<223> Synthetic peptide sequence

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Val Gly Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Ile Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Tyr Arg Ser Thr Gly Val Pro Asp Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Ser Phe Ile Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His His Tyr Ser Ala Pro Trp
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 22
<211> 321
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic nucleotide sequence

<400> 22
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atcacctgta aggccagtca ggatgtaggt actgctgtag cctggtatca gcagatccct 120

ggcaaggctc ccaagctgct gatctactcg gcatcctacc ggtccactgg cgtgcctgac 180

agattctccg gctctggctc tggcaccgat ttctccttta tcatctcctc cctccagcct 240

gaggatttcg ccacctacta ctgccagcac cattatagtg ctccgtggac gtttggcggc 300

ggaacaaagg tggagatcaa g 321

<210> 23
<211> 122
<212> PRT
<213> Mus musculus

<400> 23

Glu Val Gln Leu Val Glu Ser Gly Gly Asp Leu Val Arg Pro Gly Gly
1 5 10 15

Ser Leu Gln Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Asn
20 25 30

Gly Met Ser Trp Val Arg Gln Thr Pro Asp Arg Arg Leu Glu Trp Val
35 40 45

Ala Thr Val Ser Ser Gly Gly Ser Tyr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

[0008]

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Arg Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Arg Gln Gly Thr Thr Ala Leu Ala Thr Arg Phe Phe Asp Val Trp
100 105 110

Gly Ala Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 24
<211> 107
<212> PRT
<213> Mus musculus

<400> 24

Asp Ile Val Met Thr Gln Ser His Lys Phe Ile Ser Thr Ser Val Gly
1 5 10 15

Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Gly Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Ile Pro Gly Gln Ser Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Tyr Arg Ser Thr Gly Ile Pro Asp Arg Phe Thr Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Ser Phe Ile Ile Ser Ser Val Gln Ala
65 70 75 80

Glu Asp Leu Ala Leu Tyr Tyr Cys Gln His His Tyr Ser Ala Pro Trp
85 90 95

Thr Phe Gly Gly Gly Thr Thr Leu Asp Ile Lys
100 105

<210> 25
<211> 116
<212> PRT
<213> Mus musculus

<400> 25

Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln
1 5 10 15

Ser Leu Ser Ile Asn Cys Thr Val Ser Gly Phe Ser Leu Thr Lys Tyr
20 25 30

Gly Val His Trp Ile Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Gly Val Lys Trp Ala Gly Gly Ser Thr Asp Tyr Asn Ser Ala Leu Met
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Asn Lys Ser Gln Val Phe Leu
65 70 75 80

[0009]

Lys Met Asn Ser Leu Gln Ser Asp Asp Ser Ala Met Tyr Tyr Cys Ala
85 90 95

Arg Asp His Arg Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val
100 105 110

Thr Val Ser Ser
115

<210> 26
<211> 108
<212> PRT
<213> Mus musculus

<400> 26

Gln Val Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1 5 10 15

Glu Arg Val Thr Met Thr Cys Thr Ala Ser Leu Ser Val Ser Ser Thr
20 25 30

Tyr Leu His Trp Tyr His Gln Lys Pro Gly Ser Ser Pro Lys Leu Trp
35 40 45

Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu
65 70 75 80

Ala Glu Asp Ala Ala Thr Tyr Tyr Cys His Gln Tyr His Arg Ser Pro
85 90 95

Leu Thr Phe Gly Ser Gly Thr Lys Leu Glu Leu Lys
100 105

<210> 27
<211> 337
<212> PRT
<213> Macaca fascicularis

<400> 27

Asp Thr Glu Ile Lys Val Asn Pro Pro Gln Asp Phe Glu Ile Val Asp
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Pro Gly Tyr Leu Gly Tyr Leu Tyr Leu Gln Trp Gln Pro Pro Leu Ser
20 25 30

Leu Asp Asn Phe Lys Glu Cys Thr Val Glu Tyr Glu Leu Lys Tyr Arg
35 40 45

Asn Ile Gly Ser Glu Thr Trp Thr Thr Ile Ile Thr Lys Asn Leu His
50 55 60

Tyr Lys Asp Gly Phe Asp Leu Asn Lys Gly Ile Glu Ala Lys Ile His
65 70 75 80

Thr Leu Leu Pro Trp Gln Cys Thr Asn Gly Ser Glu Val Gln Ser Ser
85 90 95

[0010]

Trp Ala Glu Ala Thr Tyr Trp Ile Ser Pro Gln Gly Ile Pro Glu Thr
100 105 110

Lys Val Gln Asp Met Asp Cys Val Tyr Tyr Asn Trp Gln Tyr Leu Leu
115 120 125

Cys Ser Trp Lys Pro Gly Ile Gly Val Leu Leu Asp Thr Asn Tyr Asn
130 135 140

Leu Phe Tyr Trp Tyr Glu Gly Leu Asp Arg Ala Leu Gln Cys Val Asp
145 150 155 160

Tyr Ile Lys Val Asp Gly Gln Asn Ile Gly Cys Arg Phe Pro Tyr Leu
165 170 175

Glu Ser Ser Asp Tyr Lys Asp Phe Tyr Ile Cys Val Asn Gly Ser Ser
180 185 190

Glu Thr Lys Pro Ile Arg Ser Ser Tyr Phe Thr Phe Gln Leu Gln Asn
195 200 205

Ile Val Lys Pro Leu Pro Pro Val Cys Leu Thr Cys Thr Gln Glu Ser
210 215 220

Leu Tyr Glu Ile Lys Leu Lys Trp Ser Ile Pro Leu Gly Pro Ile Pro
225 230 235 240

Ala Arg Cys Phe Val Tyr Glu Ile Glu Ile Arg Glu Asp Asp Thr Thr
245 250 255

Leu Val Thr Thr Thr Val Glu Asn Glu Thr Tyr Thr Leu Lys Ile Thr
260 265 270

Asn Glu Thr Arg Gln Leu Cys Phe Val Val Arg Ser Lys Val Asn Ile
275 280 285

Tyr Cys Ser Asp Asp Gly Ile Trp Ser Glu Trp Ser Asp Lys Gln Cys
290 295 300

Trp Glu Val Glu Glu Leu Leu Lys Lys Thr Leu Leu Leu Phe Leu Leu
305 310 315 320

Pro Phe Gly Phe Ile Leu Ile Leu Val Ile Phe Val Thr Gly Leu Leu
325 330 335

Leu

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<211> 452
<212> PRT
<213> Artificial Sequence

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[0011]

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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Asn
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Thr Val Ser Ser Gly Gly Ser Tyr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gln Gly Thr Thr Ala Leu Ala Thr Arg Phe Phe Asp Val Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
115 120 125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
130 135 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145 150 155 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
165 170 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
180 185 190

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
195 200 205

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
210 215 220

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
225 230 235 240

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
245 250 255

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
260 265 270

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
275 280 285

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
290 295 300

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
305 310 315 320

[0012]

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
325 330 335

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
340 345 350

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
355 360 365

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
370 375 380

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
385 390 395 400

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
405 410 415

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
420 425 430

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Cys
435 440 445

Ser Pro Gly Lys
450

<210> 29
<211> 452
<212> PRT
<213> Artificial Sequence

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<223> Synthetic peptide sequence

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Asn
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Thr Val Ser Ser Gly Gly Ser Tyr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gln Gly Thr Thr Ala Leu Ala Thr Arg Phe Phe Asp Val Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro

[0013]

115 120 125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
130 135 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145 150 155 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
165 170 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
180 185 190

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
195 200 205

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
210 215 220

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
225 230 235 240

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
245 250 255

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
260 265 270

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
275 280 285

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
290 295 300

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
305 310 315 320

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
325 330 335

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Cys
340 345 350

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
355 360 365

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
370 375 380

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
385 390 395 400

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
405 410 415

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
420 425 430

[0014]

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
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Ser Pro Gly Lys
450

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<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 30

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Val Gly Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Tyr Arg Ser Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His His Tyr Ser Ala Pro Trp
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Cys
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 31

[0015]

<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 31

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Val Gly Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Tyr Arg Ser Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His His Tyr Ser Ala Pro Trp
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Cys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 32
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 32

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

[0016]

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Val Gly Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Tyr Arg Ser Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His His Tyr Ser Ala Pro Trp
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Cys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 33
<211> 452
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 33

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Asn
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Thr Val Ser Ser Gly Gly Ser Tyr Ile Tyr Tyr Ala Asp Ser Val

[0017]

50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gln Gly Thr Thr Ala Leu Ala Thr Arg Phe Phe Asp Val Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
115 120 125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
130 135 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145 150 155 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
165 170 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
180 185 190

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
195 200 205

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
210 215 220

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
225 230 235 240

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
245 250 255

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
260 265 270

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
275 280 285

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
290 295 300

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
305 310 315 320

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
325 330 335

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
340 345 350

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
355 360 365

[0018]

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
370 375 380

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Cys Thr Thr Pro
385 390 395 400

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
405 410 415

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
420 425 430

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Cys
435 440 445

Ser Pro Gly Lys
450

<210> 34
<211> 452
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 34

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Asn
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Thr Val Ser Ser Gly Gly Ser Tyr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gln Gly Thr Thr Ala Leu Ala Thr Arg Phe Phe Asp Val Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
115 120 125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
130 135 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145 150 155 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro

[0019]

165 170 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
180 185 190

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
195 200 205

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
210 215 220

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
225 230 235 240

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
245 250 255

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
260 265 270

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
275 280 285

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
290 295 300

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
305 310 315 320

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
325 330 335

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
340 345 350

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
355 360 365

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
370 375 380

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
385 390 395 400

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
405 410 415

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Cys Phe Ser Cys Ser Val
420 425 430

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Cys
435 440 445

Ser Pro Gly Lys
450

<210> 35
<211> 445

[0020]

<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 35

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Thr Lys Tyr
20 25 30

Gly Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Gly Val Lys Trp Ala Gly Gly Ser Thr Asp Tyr Asn Ser Ala Leu Met
50 55 60

Ser Arg Phe Thr Ile Ser Lys Asp Asn Ala Lys Asn Ser Leu Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Asp His Arg Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys
210 215 220

Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
225 230 235 240

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
245 250 255

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
260 265 270

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys

[0021]

275 280 285

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
290 295 300

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305 310 315 320

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
325 330 335

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
340 345 350

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
355 360 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
370 375 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
385 390 395 400

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
405 410 415

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 36
<211> 215
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 36

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Thr Ala Ser Leu Ser Val Ser Ser Thr
20 25 30

Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Leu Trp
35 40 45

Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Ser Tyr Thr Leu Thr Ile Ser Ser Leu Gln
65 70 75 80

Pro Glu Asp Phe Ala Thr Tyr Tyr Cys His Gln Tyr His Arg Ser Pro
85 90 95

[0022]

Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala
100 105 110

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
115 120 125

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
130 135 140

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145 150 155 160

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
165 170 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Ala Tyr Glu Lys His Lys Val
180 185 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
195 200 205

Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 37
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 37

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Thr Lys Tyr
20 25 30

Gly Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Gly Val Lys Trp Ala Gly Gly Ser Thr Asp Tyr Asn Ser Ala Leu Met
50 55 60

Ser Arg Leu Thr Ile Ser Arg Asp Asn Ala Lys Ser Ser Leu Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Asp His Arg Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser
115

<210> 38
<211> 116
<212> PRT

[0023]

<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 38

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Thr Lys Tyr
20 25 30

Gly Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Lys Trp Ala Gly Gly Ser Thr Asp Tyr Asn Ser Ala Leu Met
50 55 60

Ser Arg Phe Thr Ile Ser Lys Asp Asn Ala Lys Asn Ser Leu Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Asp His Arg Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser
115

<210> 39
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 39

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Thr Lys Tyr
20 25 30

Gly Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Lys Trp Ala Gly Gly Ser Thr Asp Tyr Asn Ser Ala Leu Met
50 55 60

Ser Arg Phe Thr Ile Ser Lys Asp Asn Ala Lys Asn Ser Leu Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Asp His Arg Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

[0024]

Thr Val Ser Ser
115

<210> 40
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 40

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Thr Lys Tyr
20 25 30

Gly Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Lys Trp Ala Gly Gly Ser Thr Asp Tyr Asn Ser Ala Leu Met
50 55 60

Ser Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Asp His Arg Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser
115

<210> 41
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 41

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Thr Lys Tyr
20 25 30

Gly Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Gly Val Lys Trp Ala Gly Gly Ser Thr Asp Tyr Asn Ser Ala Leu Met
50 55 60

Ser Arg Phe Thr Ile Ser Lys Asp Asn Ala Lys Asn Ser Leu Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala

[0025]

85 90 95

Arg Asp His Arg Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser
115

<210> 42
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 42

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Thr Ala Ser Leu Ser Val Ser Ser Thr
20 25 30

Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Leu Leu
35 40 45

Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Ser Phe Thr Leu Thr Ile Ser Ser Leu Gln
65 70 75 80

Pro Glu Asp Phe Ala Thr Tyr Tyr Cys His Gln Tyr His Arg Ser Pro
85 90 95

Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 43
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 43

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Thr Ala Ser Leu Ser Val Ser Ser Thr
20 25 30

Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Trp
35 40 45

Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln
65 70 75 80

[0026]

Pro Glu Asp Phe Ala Thr Tyr Tyr Cys His Gln Tyr His Arg Ser Pro
85 90 95

Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 44
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 44

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Thr Ala Ser Leu Ser Val Ser Ser Thr
20 25 30

Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln
65 70 75 80

Pro Glu Asp Phe Ala Thr Tyr Tyr Cys His Gln Tyr His Arg Ser Pro
85 90 95

Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 45
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 45

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Thr Ala Ser Leu Ser Val Ser Ser Thr
20 25 30

Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Trp
35 40 45

Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln
65 70 75 80

[0027]

Pro Glu Asp Phe Ala Thr Tyr Tyr Cys His Gln Tyr His Arg Ser Pro
85 90 95

Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 46
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 46

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Thr Ala Ser Leu Ser Val Ser Ser Thr
20 25 30

Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Leu Trp
35 40 45

Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Ser Phe Thr Leu Thr Ile Ser Ser Leu Gln
65 70 75 80

Pro Glu Asp Phe Ala Thr Tyr Tyr Cys His Gln Tyr His Arg Ser Pro
85 90 95

Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 47
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 47

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Thr Ala Ser Leu Ser Val Ser Ser Thr
20 25 30

Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Leu Leu
35 40 45

Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Ser Tyr Thr Leu Thr Ile Ser Ser Leu Gln
65 70 75 80

[0028]

Pro Glu Asp Phe Ala Thr Tyr Tyr Cys His Gln Tyr His Arg Ser Pro
85 90 95

Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 48
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 48

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Thr Ala Ser Leu Ser Val Ser Ser Thr
20 25 30

Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Leu Trp
35 40 45

Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Ser Tyr Thr Leu Thr Ile Ser Ser Leu Gln
65 70 75 80

Pro Glu Asp Phe Ala Thr Tyr Tyr Cys His Gln Tyr His Arg Ser Pro
85 90 95

Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 49
<211> 330
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 49

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys

[0029]

85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
100 105 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
115 120 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
130 135 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145 150 155 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
165 170 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
180 185 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
195 200 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
210 215 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
225 230 235 240

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
245 250 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
260 265 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
275 280 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
290 295 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305 310 315 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
325 330

<210> 50
<211> 452
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 50

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

[0030]

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Asn
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Thr Val Ser Ser Gly Gly Ser Tyr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gln Gly Thr Thr Ala Leu Ala Thr Arg Phe Phe Asp Val Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
115 120 125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
130 135 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145 150 155 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
165 170 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
180 185 190

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
195 200 205

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
210 215 220

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
225 230 235 240

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
245 250 255

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
260 265 270

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
275 280 285

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
290 295 300

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
305 310 315 320

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro

[0031]

325 330 335

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
340 345 350

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
355 360 365

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
370 375 380

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
385 390 395 400

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
405 410 415

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
420 425 430

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
435 440 445

Ser Pro Gly Lys
450

<210> 51
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide sequence

<400> 51

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Val Gly Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Tyr Arg Ser Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His His Tyr Ser Ala Pro Trp
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

[0032]

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Ala Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 52
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Mutant version of human constant kappa domain

<400> 52

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
1 5 10 15

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
20 25 30

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
35 40 45

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
50 55 60

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Ala Tyr Glu Lys
65 70 75 80

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
85 90 95

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
100 105

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<212> PRT
<213> Artificial Sequence

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<220>
<221> MISC FEATURE
<222> (45)..(45)
<223> Xaa at position 45 is Ala or Val

<220>

[0033]

<221> MISC_FEATURE
<222> (77)..(77)
<223> Xaa at position 77 is Ala, Gly, Ile, Val, Leu, Arg, Ser, Thr, Gln, Pro, Asn, Met, His or Trp

<220>
<221> MISC_FEATURE
<222> (83)..(83)
<223> Xaa at position 83 is Leu or Val

<400> 53

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
1 5 10 15

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
20 25 30

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Xaa Leu Gln Ser
35 40 45

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
50 55 60

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Xaa Tyr Glu Lys
65 70 75 80

His Lys Xaa Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
85 90 95

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
100 105

<210> 54
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Mutant version of human constant kappa domain

<220>
<221> MISC_FEATURE
<222> (77)..(77)
<223> Xaa at position 77 is Ala, Gly, Ile, Val, Leu, Arg, Ser, Thr, Gln, Pro, Asn, Met, His or Trp

<400> 54

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
1 5 10 15

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
20 25 30

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
35 40 45

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
50 55 60

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Xaa Tyr Glu Lys
65 70 75 80

[0034]

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
85 90 95

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
100 105

<210> 55
<211> 106
<212> PRT
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<223> Mutant version of human constant kappa domain

<400> 55

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
1 5 10 15

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
20 25 30

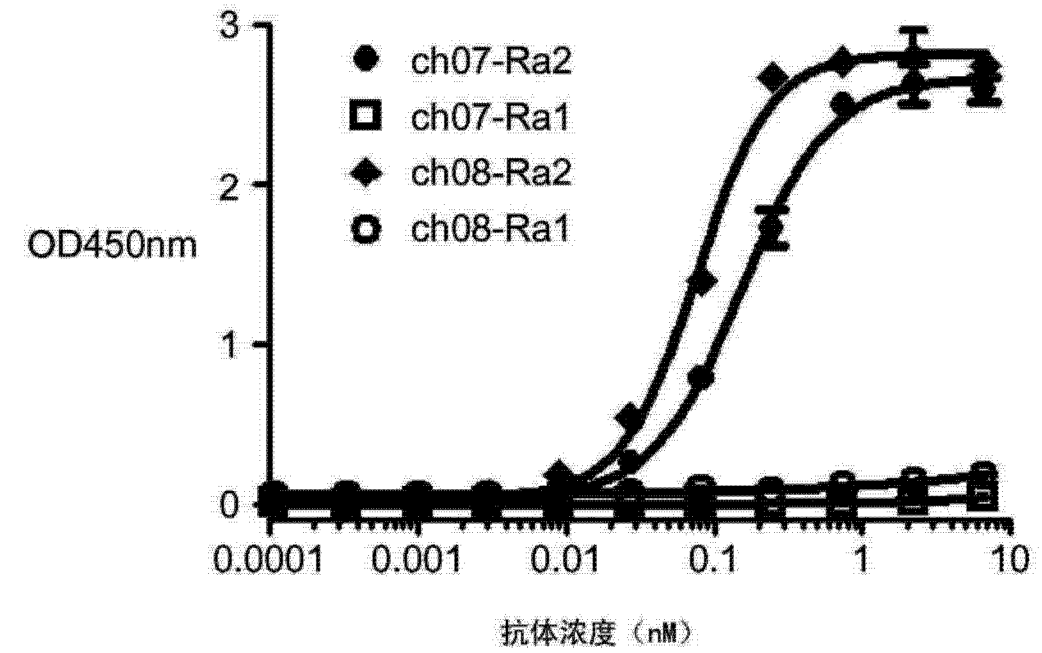
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
35 40 45

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
50 55 60

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
65 70 75 80

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
85 90 95

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
100 105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0
OD450nm
ch07-Ra2
ch07-Ra1
ch08-Ra2
ch08-Ra1
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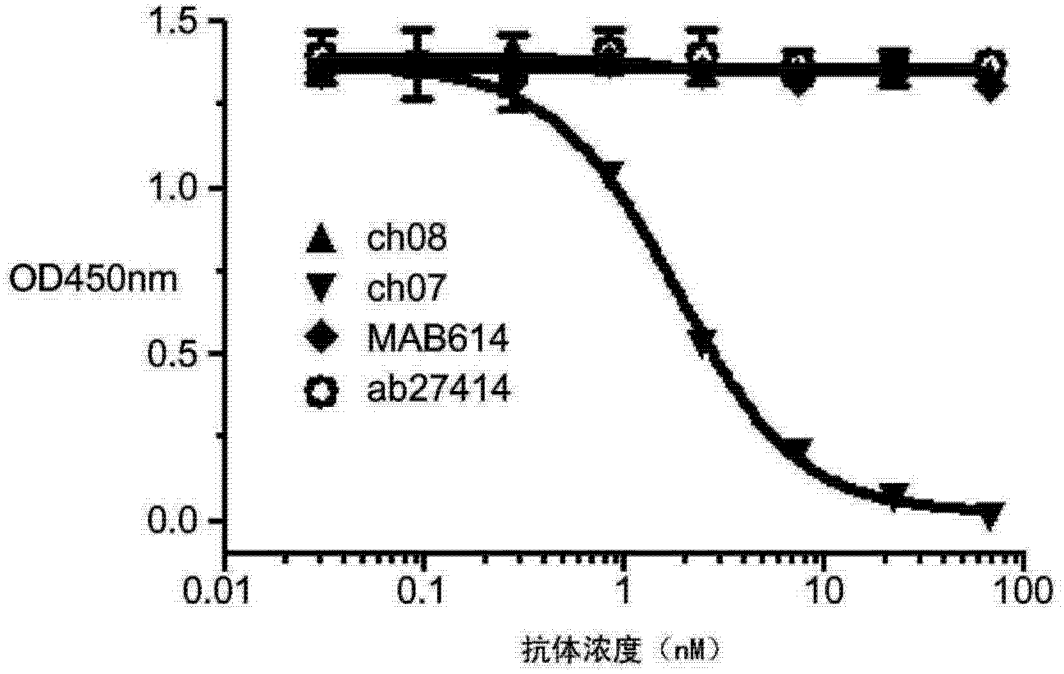
0.001
0.01
0.1
1
10
抗体浓度（nM）

图 1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0.5
0.0
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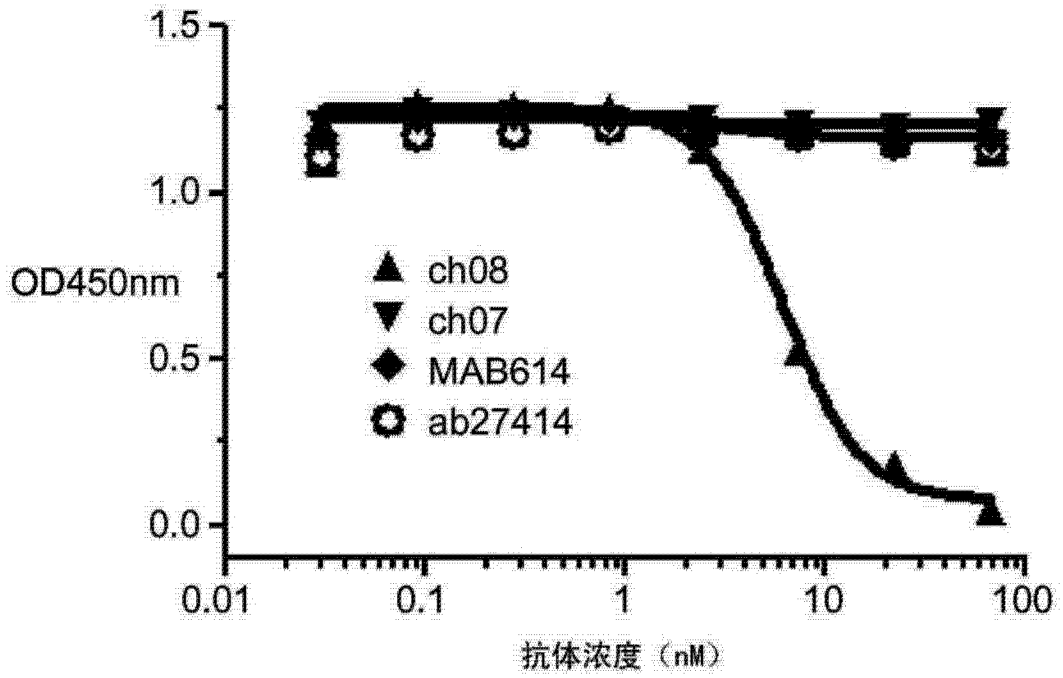
ch08
ch07
MAB614
ab27414
0.01
0.1
1
10
100
抗体浓度（nM）

图 2A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1.0
0.5
0.0
OD450nm
ch08
ch07
MAB614
ab27414
0.01
0.1
1
10
100
抗体浓度（nM）

图 2B

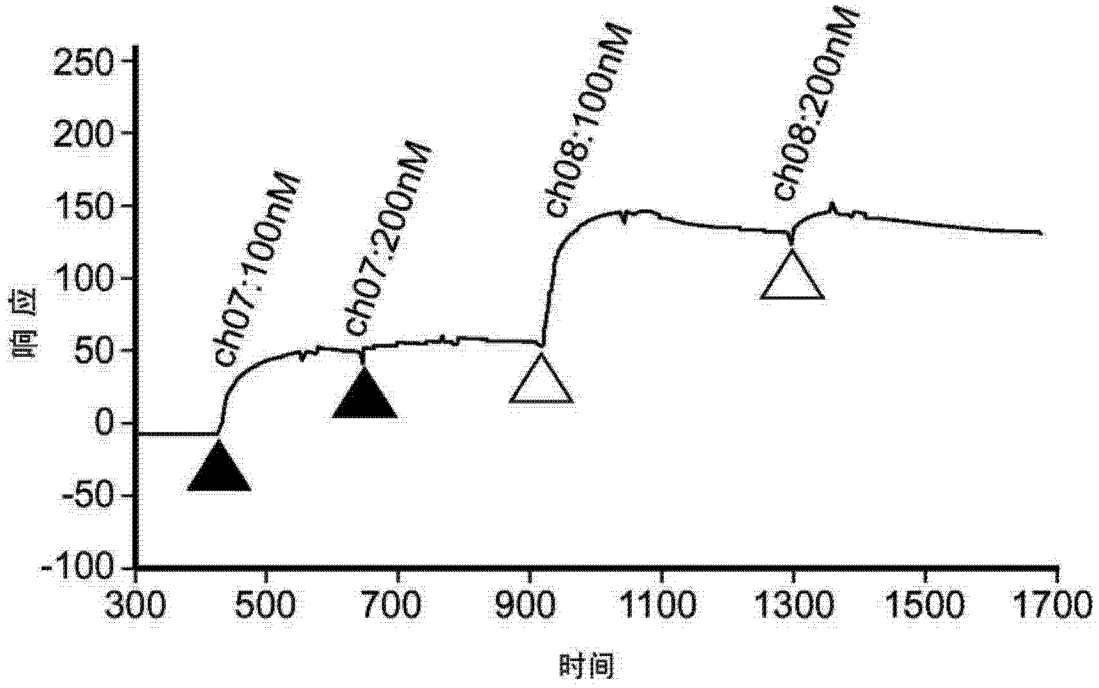
250
200
150
100
50
0
-50
-100
响应
ch07:100nM
ch07:200nM
ch08:100nM
ch08:200nM
300
500
700
900
1100
1300
1500
1700
时间

图 2C

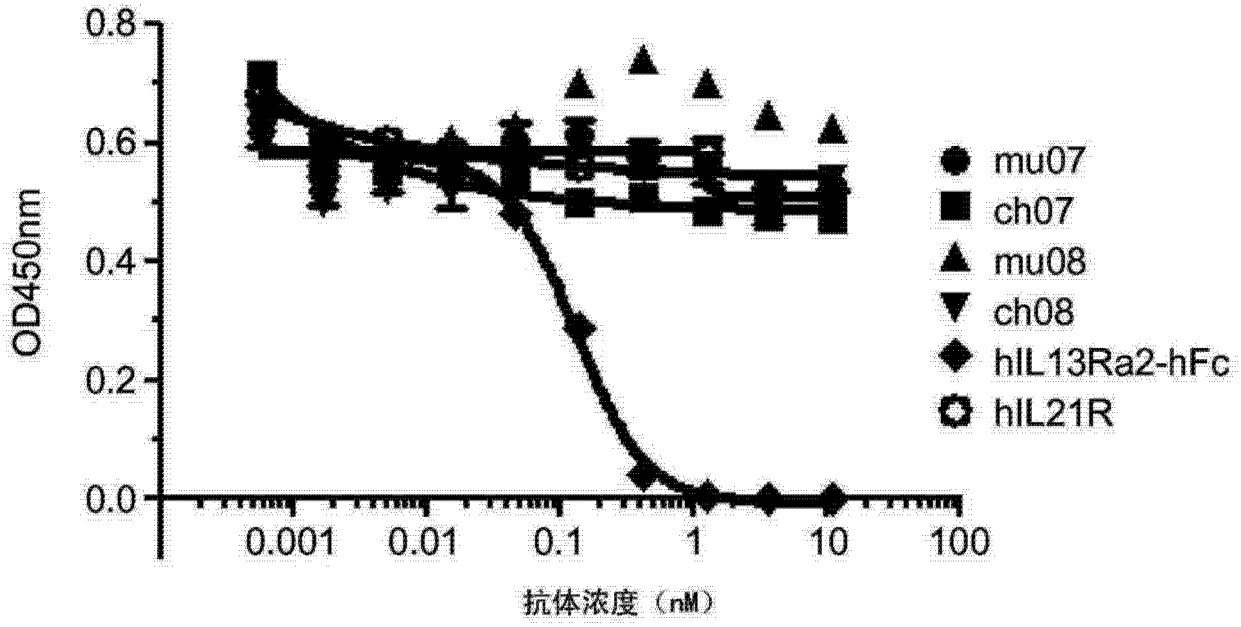
0.8
0.6
0.4
0.2
0.0
OD450nm
0.001
0.01
0.1
1
10
100
抗体浓度（nM）
mu07
ch07
mu08
ch08
hIL13Ra2-hFc
hIL21R

图 3

SEQ ID NO: 1 hu08-VH v1.0

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRNGMSWVRQAPGKGLEWVATVSSGGSYIYYADSVKGRFTISRDNAK
NSLYLQMNSLRAEDTAVYYCARQGTTALATRFFDVWGQGTLVTVSS

SEQ ID NO: 2 hu08 HCDR1

SRNGMS

SEQ ID NO: 3 hu08 HCDR2

TVSSGGSYIYYADSVKG

SEQ ID NO: 4 hu08 HCDR3

QGTTALATRFFDV

SEQ ID NO: 5 hu08-VL v1.0

DIQMTQSPSSLSASVGDRVTITCKASQDVGTAVAWYQQKPGKAPKLLIYSASYRSTGVPSRFSGSGSGTDFTLTISSL
QPEDFATYYCQHHYSAPWTFGGGTKVEIK

SEQ ID NO: 6 hu08 LCDR1

KASQDVGTAVA

SEQ ID NO: 7 hu08 LCDR2

SASYRST

SEQ ID NO: 8 hu08 LCDR3

QHHYSAPWT

SEQ ID NO: 9 hu07-VH v1.0

EVQLVESGGGLVQPGGSLRLSCAASGFTFTKYGVHWVRQAPGKGLEWVAVKWAGGSTDYNSALMSRFTISRDNAK
NSLYLQMNSLRAEDTAVYYCARDHRDAMDYWGQGTLVTVSS

SEQ ID NO: 10 hu07 HCDR1

TKYGVH

SEQ ID NO: 11 hu07 HCDR2

VKWAGGSTDYNSALMS

SEQ ID NO: 12 hu07 HCDR3

DHRDAMDY

SEQ ID NO: 13 hu07-VL v1.0

DIQMTQSPSSLSASVGDRVTITCTASLSVSSTYLHWYQQKPGKAPKLLIYSTSNLASGVPSRFSGSGSGTDFTLTISSL
QPEDFATYYCHQYHRSPLTFGGGTKVEIK

SEQ ID NO: 14 hu07 LCDR1

TASLSVSSTYLH

SEQ ID NO: 15 hu07 LCDR2

STSNLAS

SEQ ID NO: 16 hu07 LCDR3

HQYHRSPLT

图 4A

SEQ ID NO: 17 hu08-VH v1.0

GAGGTGCAGCTGGTGGAGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCTCTCTGAGACTGTCTTGTGCCGCC
TCCGGCTTCACCTTCAGTAGGAATGGCATGTCTTGGGTGAGGCAGGCCCCTGGCAAGGGCCTGGAGTGGGTGG
CCACCGTTAGTAGTGGTGGTAGTTACATCTACTATGCAGACAGTGTGAAGGGGCGGTTCACCATCTCCAGGGAC
AACGCCAAGAACTCCCTGTACCTCCAGATGAACTCCCTGAGGGCCGAGGATACCGCCGTGTACTACTGTGCCA
GACAAGGGACTACGGCACTAGCTACGAGGTTCTTCGATGTCTGGGGCCAGGGCACCCTGGTGACCGTGTCCTC
T

SEQ ID NO: 18 hu08-VL v1.0

GACATCCAGATGACCCAGTCCCCCTCTTCTCTGTCTGCCTCTGTGGGCGACAGAGTGACCATCACCTGTAAGGC
CAGTCAGGATGTAGGTACTGCTGTAGCCTGGTATCAGCAGAAGCCTGGCAAGGCTCCCAAGCTGCTGATCTACT
CGGCATCCTACCGGTCCACTGGCGTGCCTTCCAGATTCTCCGGCTCTGGCTCTGGCACCGATTTCACCCTGACC
ATCTCCTCCCTCCAGCCTGAGGATTTCGCCACCTACTACTGCCAGCACCATTATAGTGCTCCGTGGACGTTTGG
CGGCGGAACAAAGGTGGAGATCAAG

SEQ ID NO: 19 hu08-VH v1.1

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRNGMSWVRQ**T**P**D**K**R**LEWVATVSSGGSYIYYADSVKGRFTISRDNAK
NSLYLQM**S**SLRAEDTAVYYCARQGTTALATRFFDVWGQGTLVTVSS

SEQ ID NO: 20 hu08-VH v1.1

GAGGTGCAGCTGGTGGAGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCTCTCTGAGACTGTCTTGTGCCGCC
TCCGGCTTCACCTTCAGTAGGAATGGCATGTCTTGGGTGAGGCAGACCCCTGACAAGCGCCTGGAGTGGGTGG
CCACCGTTAGTAGTGGTGGTAGTTACATCTACTATGCAGACAGTGTGAAGGGGCGGTTCACCATCTCCAGGGAC
AACGCCAAGAACTCCCTGTACCTCCAGATGAGCTCCCTGAGGGCCGAGGATACCGCCGTGTACTACTGTGCCA
GACAAGGGACTACGGCACTAGCTACGAGGTTCTTCGATGTCTGGGGCCAGGGCACCCTGGTGACCGTGTCCTC
T

SEQ ID NO: 21 hu08-VL v1.1

DIQM**T**QSPSSLSASVGDRVTITCKASQDVGTAVAWYQQ**I**PGKAPKLLIYSASYRSTGVP**D**RFSGSGSGTDF**S**FIISSLQ
PEDFATYYCQHHYSAPWTFGGGTKVEIK

SEQ ID NO: 22 hu08-VL v1.1

GACATCCAGATGACCCAGTCCCCCTCTTCTCTGTCTGCCTCTGTGGGCGACAGAGTGACCATCACCTGTAAGGC
CAGTCAGGATGTAGGTACTGCTGTAGCCTGGTATCAGCAGATCCCTGGCAAGGCTCCCAAGCTGCTGATCTACT
CGGCATCCTACCGGTCCACTGGCGTGCCTGACAGATTCTCCGGCTCTGGCTCTGGCACCGATTTCTCCTTTATC
ATCTCCTCCCTCCAGCCTGAGGATTTCGCCACCTACTACTGCCAGCACCATTATAGTGCTCCGTGGACGTTTGG
CGGCGGAACAAAGGTGGAGATCAAG

图 4B

SEQ ID NO: 23 mu08 VH

EVQLVESGGDLVRPGGSLQLSCAASGFTFSRNGMSWVRQTPDRRLEWVATVSSGGSYIYYADSVKGRFTISRDNAR
NTLYLQMSSLKSEDTAMYYCARQGTTALATRFFDVWGAGTTVTVSS

SEQ ID NO: 24 mu08 VL

DIVMTQSHKFISTSVGDRVSITCKASQDVGTAVAWYQQIPGQSPKLLIYSASYRST
GIPDRFTGSGSGTDFSFIISSVQAEDLALYYCQHHYSAPWTFGGGTTLDIK

SEQ ID NO: 25 mu07 VH

QVQLKESGPGLVAPSQSLSINCTVSGFSLTKYGVHWIRQSPGKGLEWLGVKWAGGSTDYNSALMSRLTISKDNNKS
QVFLKMNSLQSDDSAMYYCARDHRDAMDYWGQGTSVTVSS

SEQ ID NO: 26 mu07 VL

QVVLTQSPAIMSASPGERVTMTCTASLSVSSTYLHWYHQKPGSSPKLWIYSTSNLASGVPARFSGSGSGTSYSLTISS
MEAEDAATYYCHQYHRSPLTFGSGTKLELK

SEQ ID NO: 27 cyno IL-13Ra2

DTEIKVNPPQDFEIVDPGYLGYLYLQWQPPLSLDNFKECTVEYELKYRNIGSETWTTIITKNLHYKDGFDLNKGIEAKIH
TLLPWQCTNGSEVQSSWAEATYWISPQGIPETKVQDMDCVYYNWQYLLCSWKPGIGVLLDTNYNLFYWYEGLDRAL
QCVDYIKVDGQNIGCRFPYLESSDYKDFYICVNGSSETKPIRSSYFTFQLQNIVKPLPPVCLTCTQESLYEIKLKWSIPL
GPIPARCFVYEIEIREDDTTLVTTTVENETYTLKITNETRQLCFVVRSKVNIYCSDDGIWSEWSDKQCWEVEELLKKTLL
LFLLPFGFILILVIFVTGLLL

SEQ ID NO: 28 hu08 HC 1.0 L443C

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRNGMSWVRQAPGKGLEWVATVSSGGSYIYYADSVKGRFTISRDNAK
NSLYLQMNSLRAEDTAVYYCARQGTTALATRFFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSCSPGK

图 4C

SEQ ID NO: 29 hu08 HC 1.0 Q347C

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRNGMSWVRQAPGKGLEWVATVSSGGSYIYYADSVKGRFTISRDNAK
NSLYLQMNSLRAEDTAVYYCARQGTTALATRFFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP**C**VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS**L**SPGK

SEQ ID NO: 30 hu08 KC 1.0 kA111C

DIQMTQSPSSLSASVGDRVTITCKASQDVGTAVAWYQQKPGKAPKLLIYSASYRSTGVPSRFSGSGSGTDFTLTISSL
QPEDFATYYCQHHYSAPWTFGGGTKVEIKRTVA**C**PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 31 hu08 KC 1.0 kK183C

DIQMTQSPSSLSASVGDRVTITCKASQDVGTAVAWYQQKPGKAPKLLIYSASYRSTGVPSRFSGSGSGTDFTLTISSL
QPEDFATYYCQHHYSAPWTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLS**C**ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 32 hu08 KC 1.0 kK188C

DIQMTQSPSSLSASVGDRVTITCKASQDVGTAVAWYQQKPGKAPKLLIYSASYRSTGVPSRFSGSGSGTDFTLTISSL
QPEDFATYYCQHHYSAPWTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLSKADYE**C**HKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 33 hu08 HC 1.0 L443C/K392C

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRNGMSWVRQAPGKGLEWVATVSSGGSYIYYADSVKGRFTISRDNAK
NSLYLQMNSLRAEDTAVYYCARQGTTALATRFFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNY**C**TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS**C**SPGK

SEQ ID NO: 34 hu08 HC 1.0 L443C/V422C

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRNGMSWVRQAPGKGLEWVATVSSGGSYIYYADSVKGRFTISRDNAK
NSLYLQMNSLRAEDTAVYYCARQGTTALATRFFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNY**K**TTPPVLDSDGSFFLYSKLTVDKSRWQQGN**C**FSCSVMHEALHNHYTQKSLS**C**SPGK

图 4D

SEQ ID NO: 35 hu07-HC-v1.5

EVQLVESGGGLVQPGGSLRLSCAASGF**SL**TKYGVHWVRQAPGKGLEWV**G**VKWAGGSTDYNSALMSRFTI**S**KDNAK
NSLYLQMNSLRAEDTAVYYCARDHRDAMDYWGQGTLVTVSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP
APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 36 hu07-LC-v1.7

DIQMTQSPSSLSASVGDRVTITC**T**ASLSVSSTYLHWYQQKPG**SS**PKL**W**IY**S**TSNLASGVPSRFSGSGSGT**SY**TLTISS
LQPEDFATYYCHQYHRSPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL
QSGNSQESVTEQDSKDSTYSLSSTLTLSKAAYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 37 hu07 VH 1.1

EVQLVESGGGLVQPGGSLRLSCAASGF**SL**TKYGVHWVRQAPGKGLEWV**G**VKWAGGSTDYNSALMS**RL**TISRDNAK
SSLYLQMNSLRAEDTAVYYCARDHRDAMDYWGQGTLVTVSS

SEQ ID NO: 38 hu07 VH 1.2

EVQLVESGGGLVQPGGSLRLSCAASGFTFTKYGVHWVRQAPGKGLEWVAVKWAGGSTDYNSALMSRFTI**S**KDNAK
NSLYLQMNSLRAEDTAVYYCARDHRDAMDYWGQGTLVTVSS

SEQ ID NO: 39 hu07 VH 1.3

EVQLVESGGGLVQPGGSLRLSCAASGF**SL**TKYGVHWVRQAPGKGLEWVAVKWAGGSTDYNSALMSRFTI**S**KDNAK
NSLYLQMNSLRAEDTAVYYCARDHRDAMDYWGQGTLVTVSS

SEQ ID NO: 40 hu07 VH 1.4

EVQLVESGGGLVQPGGSLRLSCAASGF**SL**TKYGVHWVRQAPGKGLEWVAVKWAGGSTDYNSALMSRFTISRDNAK
NSLYLQMNSLRAEDTAVYYCARDHRDAMDYWGQGTLVTVSS

SEQ ID NO: 41 hu07 VH 1.5

EVQLVESGGGLVQPGGSLRLSCAASGF**SL**TKYGVHWVRQAPGKGLEWV**G**VKWAGGSTDYNSALMSRFTI**S**KDNAK
NSLYLQMNSLRAEDTAVYYCARDHRDAMDYWGQGTLVTVSS

图 4E

SEQ ID NO: 42 hu07 VL 1.1

DIQMTQSPSSLSASVGDRVTITCTASLSVSSTYLHWYQQKPG**SS**PKLLIYSTSNLASGVPSRFSGSGSGT**S**FTLTISSL QPEDFATYYCHQYHRSPLTFGGGTKVEIK

SEQ ID NO: 43 hu07 VL 1.2

DIQMTQSPSSLSASVGDRVTITCTASLSVSSTYLHWYQQKPGKAPKL**W**IYSTSNLASGVPSRFSGSGSGTDFTLTISS LQPEDFATYYCHQYHRSPLTFGGGTKVEIK

SEQ ID NO: 44 hu07 VL 1.3

DIQMTQSPSSLSASVGDRVTITCTASLSVSSTYLHWYQQKPGKAPKLLIYSTSNLASGVPSRFSGSGSGTD**Y**TLTISSL QPEDFATYYCHQYHRSPLTFGGGTKVEIK

SEQ ID NO: 45 hu07 VL 1.4

DIQMTQSPSSLSASVGDRVTITCTASLSVSSTYLHWYQQKPGKAPKL**W**IYSTSNLASGVPSRFSGSGSGTD**Y**TLTISS LQPEDFATYYCHQYHRSPLTFGGGTKVEIK

SEQ ID NO: 46 hu07 VL 1.5

DIQMTQSPSSLSASVGDRVTITCTASLSVSSTYLHWYQQKPG**SS**PKL**W**IYSTSNLASGVPSRFSGSGSGT**S**FTLTISSL QPEDFATYYCHQYHRSPLTFGGGTKVEIK

SEQ ID NO: 47 hu07 VL 1.6

DIQMTQSPSSLSASVGDRVTITCTASLSVSSTYLHWYQQKPG**SS**PKLLIYSTSNLASGVPSRFSGSGSGT**SY**TLTISSL QPEDFATYYCHQYHRSPLTFGGGTKVEIK

SEQ ID NO: 48 hu07 VL 1.7

DIQMTQSPSSLSASVGDRVTITCTASLSVSSTYLHWYQQKPG**SS**PKL**W**IYSTSNLASGVPSRFSGSGSGT**SY**TLTISS LQPEDFATYYCHQYHRSPLTFGGGTKVEIK

SEQ ID NO:49 hu08 重链恒定区

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK

图 4F

SEQ ID NO: 50 hu08-HC v1.0

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRNGMSWVRQAPGKGLEWVATVSSGGSYIYYADSVKGRFTISRDNAK
NSLYLQMNSLRAEDTAVYYCARQGTTALATRFFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 51 hu08 LC v1.0

DIQMTQSPSSLSASVGDRVTITCKASQDVGTAVAWYQQKPGKAPKLLIYSASYRSTGVPSRFSGSGSGTDFTLTISSL
QPEDFATYYCQHHYSAPWTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLSKAAYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 52 hu08 LC 恒定区 Km(3) D77A 突变体

TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAAY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 53 hu08 LC 恒定区 45,77,83 种

TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNxLQSGNSQESVTEQDSKDSTYSLSSTLTLSKAxY
EKHKxYACEVTHQGLSSPVTKSFNRGEC

其中位置45的x是A或V，位置77的x选自A、G、I、V、L、R、S、T、Q、P、N、M、H和W，位置83的x是L或V。

SEQ ID NO: 54 hu08 LC 恒定区 Km(3) D77种突变

TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAxY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

其中位置77的x选自A、G、I、V、L、R、S、T、Q、P、N、M、H和W。

SEQ ID NO: 55 hu08 LC 恒定区 Km(3) 野生型

TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAD
YEKHKVYACEVTHQGLSSPVTKSFNRGEC

图 4G

Abstract

Disclosed herein are anti-IL-13-Rα2 antibodies and antibody drug conjugates and methods for preparing and using the same.