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(71) Applicant: **JANSSEN BIOTECH, INC.** [US/US];
800/850 Ridgeview Drive, Horsham, Pennsylvania 19044
(US).

(72) Inventors: **DUDKIN, Vadim**; 1400 McKean Road, Spring House, Pennsylvania 19477 (US). **GOLDBERG, Shalom**; 1400 McKean Road, Spring House, Pennsylvania 19477 (US). **ERHARDT, Joseph**; 1400 McKean Road, Spring House, Pennsylvania 19477 (US). **SALTER, Rhys**; 1400 McKean Road, Spring House, Pennsylvania 19477 (US). **MCDEVITT, Theresa, M.**; 1400 McKean Road, 41-2140, Spring House, Pennsylvania 19477 (US).

(74) Agent: **SHIRTZ, Joseph F.** et al.; JOHNSON & JOHNSON, One Johnson & Johnson Plaza, New Brunswick, New Jersey 08933 (US).

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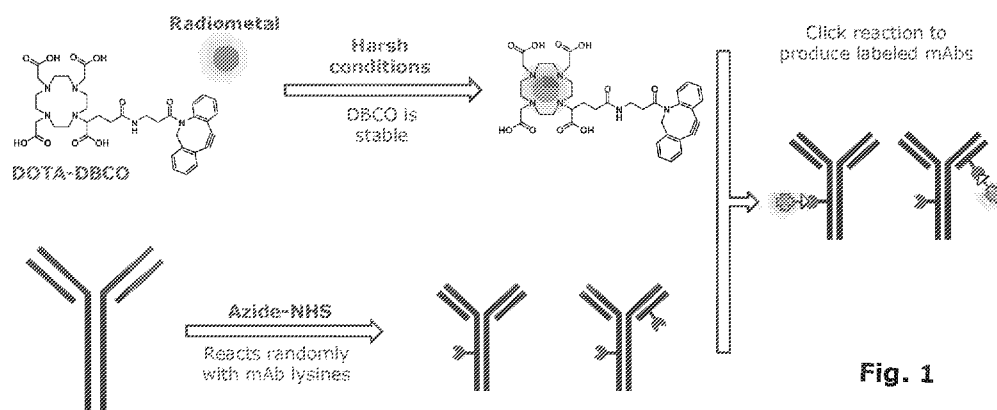


Fig. 1

(57) Abstract: Improved methods of radiolabeling antibodies using click chemistry are described. Also described are pharmaceutical compositions and uses related to the radiolabeled antibodies produced by the methods.

RADIOLABELING OF POLYPEPTIDES

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

This application contains a sequence listing, which is submitted electronically via EFS-
5 Web as an ASCII formatted sequence listing with a file name "Sequence_Listing", creation date
of December 7, 2018, and having a size of about 13.2kB. The sequence listing submitted via
EFS-Web is part of the specification and is herein incorporated by reference in its entirety.

CROSS REFERENCE TO RELATED APPLICATIONS

10 This application is entitled to priority pursuant to 35 U.S.C. § 119(e) to U.S. Provisional
Patent Application No. 62/599,830, filed on 18 December 2017, the disclosure of which is
incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

15 The invention relates to methods for radiolabeling polypeptides, such as antibodies. In
particular, the invention relates to methods of using click chemistry to label a polypeptide with a
radiometal ion. The invention also relates to pharmaceutical compositions and uses of the
radiolabeled polypeptides.

BACKGROUND OF THE INVENTION

20 Alpha particle-emitting radionuclides have great promise for cancer therapy due to their
combination of high energy with short-range action, providing the possibility of potent killing
that is mostly localized to tumor cells (Kim, Y.S. and M.W. Brechbiel, An overview of targeted
alpha therapy. *Tumour Biol*, 2012. 33(3): p. 573-90). Targeted delivery of alpha-emitters, using
25 an antibody, scaffold protein, small molecule ligand, aptamer, or other binding moiety that is
specific for a cancer antigen, provides a method of selective delivery of the radionuclide to
tumors to enhance their potency and mitigate off-target effects. In common practice, the binding
moiety is attached to a chelator which binds to the alpha-emitting radiometal to produce a

radiocomplex. Many such examples use a monoclonal antibody (mAb) as the targeting ligand, to produce what is known as a radioimmunoconjugate.

Actinium-225 (^{225}Ac) is an alpha-emitting radioisotope that is of particular interest for medical applications (Miederer et al., Realizing the potential of the Actinium-225 radionuclide generator in targeted alpha particle therapy applications. *Adv Drug Deliv Rev*, 2008. 60(12):71-82). The 10-day half-life of ^{225}Ac is long enough to facilitate radio-conjugate production, but short enough to match the circulation pharmacokinetics of delivery vehicles such as antibodies. As such, ^{225}Ac radioimmunoconjugates are of particular interest. Additionally, ^{225}Ac decays in a series of steps that ultimately emits 4 alpha particles before reaching a stable isotope, ^{209}Bi , thereby increasing the potency. Another radioisotope of interest for medical applications is Lutetium-177 (^{177}Lu), which emits both gamma-irradiation suitable for imaging and medium-energy beta-irradiation suitable for radiotherapy. It has been shown that ^{177}Lu -labeled peptides demonstrate reduced normal tissue damage, and that ^{177}Lu -labeling makes it possible to use a single radiolabeled agent for both therapy and imaging (Kwekkeboom DJ, et al. [^{177}Lu -DOTAOTyr3]octreotate: comparison with [^{111}In -DTPA]octreotide in patients. *Eur J Nucl Med*. 2001;28: p. 1319–1325). Other radioisotopes that are used for therapeutic applications include, e.g., beta or alpha emitters, such as, e.g., thorium, radium, ^{32}P , ^{47}Sc , ^{67}Cu , ^{77}As , ^{89}Sr , ^{90}Y , ^{99}Tc , ^{105}Rh , ^{109}Pd , ^{111}Ag , ^{131}I , ^{153}Sm , ^{159}Gd , ^{165}Dy , ^{166}Ho , ^{169}Er , ^{186}Re , ^{188}Re , ^{194}Ir , ^{198}Au , ^{199}Au , ^{211}At , ^{212}Pb , ^{212}Bi , ^{213}Bi , ^{223}Ra , ^{255}Fm and ^{227}Th . Other radioisotopes that are used for imaging applications include gamma-emitting radioisotopes, such as, e.g., ^{62}Cu , ^{64}Cu , ^{67}Ga , ^{68}Ga , ^{86}Y , ^{89}Zr , and ^{111}In .

Previous clinical and pre-clinical programs have largely used 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) for actinium chelation. However, it is known that DOTA chelation of actinium can be challenging (Deal, K.A., et al., Improved in vivo stability of actinium-225 macrocyclic complexes. *J Med Chem*, 1999. 42(15): p. 2988-92), and often requires either harsh conditions or high levels of DOTA per antibody. As a result, two different approaches – known as the “1-step” and “2-step” radiolabeling methods – have been utilized, each with their own drawbacks.

The “2-step” method, which includes 2 chemical steps involving actinium, was the first to be developed (McDevitt, M.R., et al., Tumor therapy with targeted atomic nanogenerators. *Science*, 2001. 294(5546): p. 1537-40). ^{225}Ac was chelated by the bifunctional chelator (BFC) DOTA-isothiocyanate (DOTA-SCN) in high radiochemical yield (~95%) at pH 4.5-5 using 2 M acetate buffer at 55° to 60°C for 30 min. Subsequently, the ^{225}Ac -DOTA-SCN was reacted with the targeting antibody to produce the radioimmunoconjugate. The major drawback of the 2-step method is that ~90% of the SCN fails to survive the labeling conditions, thus ~90% of the input ^{225}Ac is conjugated to nonreactive forms of DOTA that cannot be conjugated to the antibody. This results in not only low yield (typically only about 10%) and higher costs, but also lowered specific activity that can limit the efficacy of the final conjugate.

The “1-step” method was developed more recently for actinium (Maguire, W.F., et al., Efficient 1-step radiolabeling of monoclonal antibodies to high specific activity with ^{225}Ac for alpha-particle radioimmunotherapy of cancer. *J Nucl Med*, 2014. 55(9): p. 1492-8). This method has only 1 chemical reaction step involving actinium. The DOTA-SCN was first conjugated to the antibody. The ^{225}Ac was then chelated to the DOTA-mAb under mild conditions (37°C, pH 7.5), achieving up to 80% radiochemical yield. However, it was necessary to conjugate high levels of DOTA (~10 or more per antibody) to achieve high yields. The high chelator:antibody ratio (CAR), in this case the high DOTA:Ab ratio (DAR), species are more likely to have compromised immunoreactivity; furthermore, though the average DAR may be 10, it is likely that the ^{225}Ac is chelating to populations with ratios even higher than the average. Thus, this method runs the risk of linking the ^{225}Ac to the least active fraction of antibody-chelator conjugates. Furthermore, it is necessary to handle the antibody and DOTA-mAb conjugate under metal-free conditions to avoid chelation of common metals such as iron, zinc and copper, which introduces significant challenges into the production process.

Click chemistry is a chemical approach introduced by Sharpless in 2001 and describes chemistry tailored to generate substances quickly and reliably by joining small units together. See, e.g., Kolb, Finn and Sharpless *Angewandte Chemie International Edition* (2001) 40: 2004-2021; Evans, *Australian Journal of Chemistry* (2007) 60: 384-395). Coupling reactions (some of which may be classified as “click chemistry”) include, but are not limited to, formation of esters,

thioesters, amides (e.g., such as peptide coupling) from activated acids or acyl halides; nucleophilic displacement reactions (e.g., such as nucleophilic displacement of a halide or ring opening of strained ring systems); azide-alkyne Huisgen cycloaddition (e.g., 1,3-dipolar cycloaddition between an azide and an alkyne to form a 1,2,3-triazole linker); thiol-alkyne addition; imine formation; Diels-Alder reactions between tetrazines and trans-cyclooctene (TCO); and Michael additions (e.g., maleimide addition).

Click chemistry reactions between alkynes and azides typically require the addition of a copper catalyst to promote the 1,3-cycloaddition reaction, and are known as copper-catalyzed azide-alkyne cycloaddition (CuAAC) reactions. However, click chemistry reactions between cyclooctyne or cyclooctyne derivatives and azides typically do not require the addition of a copper catalyst, and instead proceed via strain-promoted azide-alkyne cycloaddition (SPAAC) (Debets, M.F., et al., Bioconjugation with strained alkenes and alkynes. *Acc Chem Res*, 2011. 44(9): p. 805-15).

Site-specificity has become a key area of focus in the antibody-drug conjugate (ADC) field (Agarwal, P. and C.R. Bertozzi, Site-specific antibody-drug conjugates: the nexus of bioorthogonal chemistry, protein engineering, and drug development. *Bioconjug Chem*, 2015. 26(2): p. 176-92), as it has been demonstrated that both efficacy and safety of ADCs can be increased with site-specific methods as compared to random conjugation. It is thought that similar safety and efficacy benefits could be achieved for radioimmunoconjugates.

As indicated above, there remains a need in the art for efficient methods of producing stable radioimmunoconjugates with high specific activity and high yield.

BRIEF SUMMARY OF THE INVENTION

The invention satisfies this need by providing methods for using click chemistry to radiolabel polypeptides, such as antibodies. In a method of the invention, an azide-modified antibody and a radiocomplex comprising a radiometal ion associated with a chelating moiety comprising an alkyne group are used in a click chemistry reaction to produce stable radioimmunoconjugates having a low chelator:antibody ratio (CAR) and high radiochemical yields, while requiring reduced usage of the radioactive metal and only requiring metal-free

conditions in the step used to produce the initial radiocomplex. Methods of the invention simplify previous methods for producing radioimmunoconjugates with increased safety, efficacy and uniformity.

In one general aspect, the invention relates to a method of labeling a polypeptide with a radiometal ion, the method comprising:

- a. providing a modified polypeptide comprising the polypeptide covalently linked to a first click reaction partner;
- b. providing a radiocomplex comprising the radiometal ion associated with a chelating moiety, wherein the chelating moiety comprises a chelant covalently linked to a second click reaction partner; and
- c. contacting the modified polypeptide with the radiocomplex under a condition to allow the first click reaction partner to react with the second click reaction partner to thereby label the polypeptide with the radiometal ion.

In another general aspect, the invention relates to a pharmaceutical composition comprising a radiolabeled polypeptide prepared by a method of the invention and a pharmaceutically acceptable carrier.

In another general aspect, the invention relates to a method of treating a neoplastic disease or disorder in a subject in need thereof, comprising administering to the subject a pharmaceutical composition of the invention.

In another general aspect, the invention relates to a combination or kit comprising:

- a. a modified polypeptide comprising a polypeptide covalently linked to a first click reaction partner; and
 - b. a radiocomplex comprising a radiometal ion associated with a chelating moiety, wherein the chelating moiety comprises a chelant covalently linked to a second click reaction partner;
- wherein the combination or kit is to be used for labeling the polypeptide with the radiometal ion.

In other general aspects, the invention relates to a therapeutic or diagnostic agent (“theranostic agent”) comprising a radiolabeled polypeptide prepared by a method of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawings. It should be understood that the invention is not limited to the precise embodiments shown in the drawings.

In the drawings:

Fig. 1 shows a schematic of radiolabeling an antibody according to a method of the invention; random conjugation is shown in the figure, and a similar radiolabeling scheme is used when the azides are conjugated to the monoclonal antibody (mAb) site-specifically;

Fig. 2 shows a synthesis scheme of an improved two-step preparation of ^{89}Zr -DOTA-mAb via click chemistry according to an embodiment of the application;

Fig. 3 shows cell binding of In-111 radioimmunoconjugates: bound radioactivity increases with increasing cell number; in particular:

Fig. 3A shows the binding to the human prostate cancer cell line C4-2B (PSMA+, Transferrin receptor+) by a PSMA-binding antibody (“PSMB127”) In-111 radioimmunoconjugate and a human transferrin In-111 radioconjugate according to embodiments of the application; and

Fig. 3B shows the binding to the human epidermoid carcinoma cancer cell line A431 (EGFR+) by EGFR-binding antibodies cetuximab and panitumumab In-111 radioimmunoconjugates according to embodiments of the application, and the lack of binding of these conjugates to the control (EGFR-) human AML cell line MOLM-13;

Fig. 4 shows kinetics of cell internalization of In-111 in the human prostate cancer cell line C4-2B treated with a anti-PSMA mAb In-111 radioimmunoconjugate according to an embodiment of the application; surface-bound In-111 rapidly disappeared from the cell surface and was redistributed intracellularly; and

Fig. 5 shows the results of a mouse tumor xenograft study; mice were implanted with human prostate cancer LNCaP cells; when tumors reached 100 mm³, mice were treated with a single dose of a click radiolabeled anti-PSMA mAb (“PSMB127”) actinium radioconjugate according to an embodiment of the application at a range of activities or the isotype control, a human IgG4 antibody that binds to a viral target absent in this system radioconjugate; in particular:

Fig. 5A shows the tumor volume for each group; sizes were plotted until under half of the group remained;

Fig. 5B shows survival curves for control mAb groups; and

Fig. 5C shows survival curves for anti-PSMA mAb groups.

DETAILED DESCRIPTION OF THE INVENTION

Various publications, articles and patents are cited or described in the background and throughout the specification; each of these references is herein incorporated by reference in its entirety. Discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is for the purpose of providing context for the invention. Such discussion is not an admission that any or all of these matters form part of the prior art with respect to any inventions disclosed or claimed.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning commonly understood to one of ordinary skill in the art to which this invention pertains. Otherwise, certain terms cited herein have the meanings as set in the specification. All patents, published patent applications and publications cited herein are incorporated by reference as if set forth fully herein

It must be noted that as used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural reference unless the context clearly dictates otherwise.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising”, will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integer or step. When used herein the term

“comprising” can be substituted with the term “containing” or “including” or sometimes when used herein with the term “having”.

When used herein “consisting of” excludes any element, step, or ingredient not specified in the claim element. When used herein, “consisting essentially of” does not exclude materials or steps that do not materially affect the basic and novel characteristics of the claim. Any of the
5 aforementioned terms of “comprising”, “containing”, “including”, and “having”, whenever used herein in the context of an aspect or embodiment of the invention can be replaced with the term “consisting of” or “consisting essentially of” to vary scopes of the disclosure.

As used herein, the conjunctive term “and/or” between multiple recited elements is
10 understood as encompassing both individual and combined options. For instance, where two elements are conjoined by “and/or”, a first option refers to the applicability of the first element without the second. A second option refers to the applicability of the second element without the first. A third option refers to the applicability of the first and second elements together. Any one of these options is understood to fall within the meaning, and therefore satisfy the requirement of
15 the term “and/or” as used herein. Concurrent applicability of more than one of the options is also understood to fall within the meaning, and therefore satisfy the requirement of the term “and/or.”

In an attempt to help the reader of the application, the description has been separated into various paragraphs or sections, or is directed to various embodiments of the application. These separations should not be considered as disconnecting the substance of a paragraph or section or
20 embodiments from the substance of another paragraph or section or embodiments. To the contrary, one skilled in the art will understand that the description has broad application and encompasses all the combinations of the various sections, paragraphs and sentences that can be contemplated. The discussion of any embodiment is meant only to be exemplary and is not intended to suggest that the scope of the disclosure, including the claims, is limited to these
25 examples.

Click radiolabeling of polypeptides

In contrast to known procedures, methods of the present invention provide for an improved method for the production of radioimmunoconjugates that are suitable, for example, for medicinal applications in subjects, e.g., humans, in need thereof. In particular, methods

described herein address the major limitations of current methods by providing processes for both high yield chelation of metal ions, including but not limited to, ^{225}Ac , ^{111}In and ^{89}Zr , and for low DAR. The invention allows for the production of a single batch of azide-labeled polypeptides, such as azide-mAb conjugate that can then be used for the production of a radiolabeled diagnostic (e.g., when labeled with ^{89}Zr or ^{111}In) or therapeutic (e.g., when labeled with ^{225}Ac) purposes, in which the radiolabel is attached at the same site(s) within the batch of azide-labeled polypeptides, which can be obtained either by site-specific modification or by random azide conjugation. For example, in the case of random azide conjugation, samples of a batch of azide-labeled polypeptides comprising a single distribution of azide-modified sites can be radiolabeled for different purposes using click chemistry of the invention.

A method of the invention, which relies on click chemistry and is referred to as “click radiolabeling”, involves (1) obtaining a modified polypeptide, such as an antibody, that includes a first click chemistry reaction partner, e.g. an azide moiety; (2) obtaining a radiocomplex comprising a radiometal ion, e.g., ^{225}Ac , ^{111}In or ^{89}Zr , associated with a chelating moiety, wherein the chelating moiety comprises a chelant covalently linked to a second click chemistry reaction partner, e.g. an alkyne group, such as DOTA-dibenzocyclooctyne (DOTA-DBCO) or deferoxamine-DBCO (DFO-DBCO); and (3) conducting a reaction between the click chemistry reaction partners of the modified peptide and the radiocomplex, such as a strain-promoted alkyne-azide cycloaddition (SPAAC) between the azide moiety and the alkyne group.

A method of the invention allows chelation of the radioactive metal under low or high pH and/or high temperature conditions to maximize efficiency, which can be accomplished without the risk of inactivating the alkyne reaction partner. The efficient chelation and efficient SPAAC reaction between azido-mAb and the radiocomplex allows radioimmunoconjugates to be produced with high radiochemical yield even with low azide:mAb ratios. In a method of the invention, the only step in which trace metals must be excluded is the radiometal ion chelation to the chelating moiety; the antibody production, purification, and conjugation steps do not need to be conducted under metal free conditions.

As used herein, the term “click chemistry” refers to a chemical philosophy introduced by Sharpless, describing chemistry tailored to generate covalent bonds quickly and reliably by

joining small units comprising reactive groups together (see Kolb, et al., *supra*). Click chemistry does not refer to a specific reaction, but to a concept including, but not limited to, reactions that mimic reactions found in nature. In some embodiments, click chemistry reactions are modular, wide in scope, give high chemical yields, generate inoffensive byproducts, are stereospecific, exhibit a large thermodynamic driving force to favor a reaction with a single reaction product, and/or can be carried out under physiological conditions. In some embodiments, a click chemistry reaction exhibits high atom economy, can be carried out under simple reaction conditions, uses readily available starting materials and reagents, uses no toxic solvents or uses a solvent that is benign or easily removed, such as water, and/or provides simple product isolation by non-chromatographic methods, such as crystallization or distillation. In certain embodiments, the click chemistry reaction is a Huisgen cycloaddition or the 1,3-dipolar cycloaddition between an azide (-N₃) and an alkyne, or an alkyne moiety, to form a 1,2,4-triazole linker.

In a general aspect, the invention relates to a method of labeling a polypeptide, an aptamer, or a small molecule ligand with a radiometal ion, the method comprising:

- a. providing a modified polypeptide comprising the polypeptide covalently linked to a first click reaction partner;
- b. providing a radiocomplex comprising the radiometal ion associated with a chelating moiety, wherein the chelating moiety comprises a chelant covalently linked to a second click reaction partner; and
- c. contacting the modified polypeptide with the radiocomplex under a condition to allow the first click reaction partner to react with the second click reaction partner to thereby label the polypeptide with the radiometal ion.

As used herein, the term “polypeptide” refers to a polymer composed of amino acid residues, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof linked via peptide bonds. The term refers to a polypeptide of any size, structure, or function. Typically, a polypeptide is at least three amino acids long. A polypeptide can be naturally occurring, recombinant, or synthetic, or any combination thereof. Synthetic polypeptides can be synthesized, for example, using an automated polypeptide synthesizer. According to preferred embodiments, the polypeptide is an antibody, preferably a monoclonal

antibody, or a fragment thereof, such as an antigen-binding fragment thereof. According to preferred embodiments, the antibody or fragment thereof is specific for a cancer antigen. According to other embodiments, the polypeptide is an engineered domain or a scaffold protein.

As used herein, the term “antibody” or “immunoglobulin” is used in a broad sense and includes immunoglobulin or antibody molecules including polyclonal antibodies, monoclonal antibodies including murine, human, human-adapted, humanized and chimeric monoclonal antibodies, and antigen-binding fragments thereof.

In general, antibodies are proteins or peptide chains that exhibit binding specificity to a specific antigen, referred to herein as a “target”. Antibody structures are well known. Immunoglobulins can be assigned to five major classes, namely IgA, IgD, IgE, IgG and IgM, depending on the heavy chain constant domain amino acid sequence. IgA and IgG are further sub-classified as the isotypes IgA1, IgA2, IgG1, IgG2, IgG3 and IgG4. Accordingly, the antibodies of the invention can be of any of the five major classes or corresponding sub-classes. Preferably, the antibodies of the invention are IgG1, IgG2, IgG3 or IgG4. Antibody light chains of any vertebrate species can be assigned to one of two clearly distinct types, namely kappa and lambda, based on the amino acid sequences of their constant domains. Accordingly, the antibodies of the invention can contain a kappa or lambda light chain constant domain. According to particular embodiments, the antibodies of the invention include heavy and/or light chain constant regions from mouse antibodies or human antibodies. Each of the four IgG subclasses has different biological functions known as effector functions. These effector functions are generally mediated through interaction with the Fc receptor (FcγR) or by binding C1q and fixing complement. Binding to FcγR can lead to antibody dependent cell mediated cytotoxicity, whereas binding to complement factors can lead to complement mediated cell lysis. An antibody useful for the invention can have no or minimal effector function, but retain its ability to bind FcRn.

As used herein, the term “antigen-binding fragment” refers to an antibody fragment such as, for example, a diabody, a Fab, a Fab', a F(ab')₂, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a (dsFv)₂, a bispecific dsFv (dsFv-dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), a single domain antibody (sdab) an scFv

dimer (bivalent diabody), a multispecific antibody formed from a portion of an antibody comprising one or more CDRs, a camelized single domain antibody, a nanobody, a domain antibody, a bivalent domain antibody, or any other antibody fragment that binds to an antigen but does not comprise a complete antibody structure. An antigen-binding fragment is capable of binding to the same antigen to which the parent antibody or a parent antibody fragment binds. As used herein, the term “single-chain antibody” refers to a conventional single-chain antibody in the field, which comprises a heavy chain variable region and a light chain variable region connected by a short peptide of about 15 to about 20 amino acids. As used herein, the term “single domain antibody” refers to a conventional single domain antibody in the field, which comprises a heavy chain variable region and a heavy chain constant region or which comprises only a heavy chain variable region.

As used herein, the term “scaffold” or “scaffold protein” refers to any protein that has a target binding domain and that can bind to a target. A scaffold contains a “framework”, which is largely structural, and a “binding domain” which makes contact with the target and provides for specific binding. The binding domain of a scaffold need not be defined by one contiguous sequence of the scaffold. In certain cases, a scaffold may be part of larger binding protein, which, itself, may be part of a multimeric binding protein that contains multiple scaffolds. Certain binding proteins may be bi- or multi-specific in that they can bind to two or more different epitopes. A scaffold can be derived from a single chain antibody, or a scaffold may be not antibody-derived.

Polypeptides of the invention can be covalently linked to a first click reaction partner using any method for chemical or enzymatic modification of a polypeptide known to those skilled in the art in view of the present disclosure. Amine-reactive groups that react with primary amines that exist at the N-terminus of each polypeptide chain and in the side-chain of lysine residues can be used in methods for random modification of polypeptides. Examples of amine-reactive groups suitable for use in the invention include, but are not limited to, N-hydroxy succinimide (NHS), substituted NHS, such as sulfo-NHS, isothiocyanate, and tetra- and per-fluoro phenyl ester. Thiol-reactive groups that react with thiols, or sulfhydryls, that exist in the side-chain of cysteine residues can be used in methods for random modification of polypeptides.

Examples of thiol-reactive groups suitable for use in the invention include, but are not limited to, maleimide, haloacetyl and phenyloxadiazole sulfone. According to preferred embodiments, a modified polypeptide is obtained by reacting a side chain, preferably the amino side chain of a lysine, with an electrophile covalently linked to the first click reaction partner (e.g. NHS-azide).

5 A method of the invention further allows the production of site-specific radiolabeled polypeptides. The click radiolabeling method of the invention facilitates site-specific production of radioimmunoconjugates by taking advantage of established methods to install azide groups site-specifically on antibodies (Li, X., et al. Preparation of well-defined antibody-drug conjugates through glycan remodeling and strain-promoted azide-alkyne cycloadditions. *Angew Chem Int Ed Engl*, 2014. 53(28): p. 7179-82; Xiao, H., et al., Genetic incorporation of multiple unnatural amino acids into proteins in mammalian cells. *Angew Chem Int Ed Engl*, 2013. 52(52): p. 14080-3). Methods of attaching molecules to proteins or antibodies in a site-specific manner are known in the art, and any method of site-specifically labeling an antibody known to those skilled in the art can be used in the invention in view of the present disclosure. Examples of
10 methods to site-specifically modify antibodies suitable for use in the invention include, but are not limited to, incorporation of engineered cysteine residues (e.g., THIOMAB™), use of non-natural amino acids or glycans (e.g., seleno cysteine, p-AcPhe, formylglycine generating enzyme (FGE, SMARTag™), etc.), and enzymatic methods (e.g., use of glycotransferase, endoglycosidase, microbial or bacterial transglutaminase (MTG or BTG), sortase A, etc.).

20 According to preferred embodiments, the modified polypeptide is an antibody or antigen binding fragment thereof that is obtained by trimming the antibody or antigen binding fragment thereof with a bacterial endoglycosidase specific for the β -1,4 linkage between a core GlcNAc residue in a Fc-glycosylation site of the antibody, such as GlycINATOR (Genovis), which leaves the inner most GlcNAc intact on the Fc, allowing for the site-specific incorporation of azido sugars at that
25 site. The trimmed antibody or antigen binding fragment thereof can then be reacted with an azide-labeled sugar, such as UDP-N-azidoacetylgalactosamine (UDP-GalNaz) or UDP-6-azido 6-deoxy GalNAc, in the presence of a sugar transferase, such as GalT galactosyltransferase or GalNAc transferase, to thereby obtain the modified antibody or antigen binding fragment thereof. According to other preferred embodiments, modified polypeptide is an antibody or

antigen binding fragment thereof that is obtained by deglycosylating the antibody or antigen binding fragment thereof with an amidase. The resulting deglycosylated antibody or antigen binding fragment thereof can then be reacted with an azido amine, preferably 3-azido propylamine, 6-azido hexylamine, or any azido-linker-amine or any azido-alkyl-amine, such as
5 an azido-polyethylene glycol (PEG)-amine, for example, O-(2-Aminoethyl)-O'-(2-azidoethyl)tetraethylene glycol, O-(2-Aminoethyl)-O'-(2-azidoethyl)pentaethylene glycol, O-(2-Aminoethyl)-O'-(2-azidoethyl)triethylene glycol, etc., or in the presence of a microbial transglutaminase to thereby obtain the modified antibody or antigen binding fragment thereof.

As used herein, the term “aptamer” refers to a single-stranded oligonucleotide (single-
10 stranded DNA or RNA molecule) that can bind specifically to its target with high affinity. The aptamer can be used as a molecule targeting various organic and inorganic materials.

As used herein, the term “small molecule ligand” refers to a low molecular weight organic compound. Small molecule ligands, as used herein, can refer to compounds that have a size of less than about 1000 daltons, and can be synthesized in the laboratory or found in nature.

As used herein, the term “click reaction partner” or “click chemistry handle” refers to a
15 reactant or a reactive group that can partake in a click chemistry reaction. A click reaction partner can be a moiety that is rarely found in naturally-occurring biomolecules and is chemically inert towards biomolecules, but, e.g., when reacted with an azide-reactive or alkyne-reactive group, the reaction can take place efficiently under biologically relevant conditions, for
20 example in cell culture conditions, such as in the absence of excess heat or harsh reactants. In general, click chemistry reactions require at least two molecules comprising click reaction partners that can react with each other. Such click reaction partners that are reactive with each other are sometimes referred to herein as click chemistry handle pairs, or click chemistry pairs. In some embodiments, the click reaction partners are an azide and a strained alkyne, e.g. a
25 cyclooctyne, or any other alkyne. In other embodiments, the click reaction partners are reactive dienes and suitable tetrazine dienophiles. For example, trans-cyclooctene, norbornene, or bicyclononene can be paired with a suitable tetrazine dienophile as a click reaction pair. In yet other embodiments, tetrazoles can act as latent sources of nitrile imines, which can pair with unactivated alkenes in the presence of ultraviolet light to create a click reaction pair, termed a

“photo-click” reaction pair. In other embodiments, the click reaction partners are a cysteine and a maleimide. For example the cysteine from a peptide (e.g., GGGC) can be reacted with a maleimide that is associated with a chelating agent (e.g., NOTA). Other suitable click chemistry handles are known to those of skill in the art (see, e.g., Spicer et al., Selective chemical protein
5 modification. *Nature Communications*. 2014; 5: p. 4740). In other embodiments, the click reaction partners are Staudinger ligation components, such as phosphine and azide. In other embodiments, the click reaction partners are Diels-Alder reaction components, such as dienes, such as tetrazine, and alkenes, such as trans-cyclooctene (TCO) or norbornene. Exemplary click reaction partners are described in US20130266512 and in WO2015073746, the relevant
10 description on click reaction partners in both of which are incorporated by reference herein. According to preferred embodiments, one of the first and second click reaction partners comprises an alkyne group, and the other click reaction partner comprises an azide. According to other preferred embodiments, one of the first and second click reaction partners comprises an alkene group, and the other click reaction partner comprises a diene.

15 As used herein, the term “alkyne”, “alkyne group” or “alkyne moiety” refers to a functional group comprising a carbon-carbon triple bond. Alkyne moieties include terminal alkynes and cyclic alkynes, preferably terminal alkynes and cyclic alkynes that are reactive with azide groups. A terminal alkyne has at least one hydrogen atom bonded to a triply bonded carbon atom. A cyclic alkyne is a cycloalkyl ring comprising one or more triple bonds. Examples of
20 cyclic alkynes include, but are not limited to, cyclooctyne and cyclooctyne derivatives, such as bicyclononyne (BCN), difluorinated cyclooctyne (DIFO), dibenzocyclooctyne (DIBO), keto-DIBO, biarylazacyclooctynone (BARAC), dibenzoazacyclooctyne (DIBAC), dimethoxyazacyclooctyne (DIMAC), dibenzoycyclooctyne (DBCO), difluorobenzocyclooctyne (DIFBO), monobenzocyclooctyne (MOBO), and tetramethoxy DIBO (TMDIBO). According to
25 preferred embodiments, one of the first and second click reaction partners comprises a cyclic alkyne, preferably DBCO. According to preferred embodiments, the other click reaction partner comprises an azide, preferably NHS-azide.

As used herein, the term “diene” refers to a compound having two carbon-to-carbon double bonds where these double bonds are conjugated in the 1,3-position. The double bonds of

the diene can be either cis or trans. Examples of dienes include, but are not limited to, a tetrazine or a tetrazole group.

As used herein, the term “alkene”, “alkene group” or “alkene moiety” refers to an unsaturated hydrocarbon molecule that includes a carbon-carbon double bond. According to particular embodiments, an alkene can include from 2 to 100 carbon atoms. Examples of alkenes include, but are not limited to, norbornene and trans-cyclooctene (TCO). According to other preferred embodiments, one of the first and second click reaction partners comprises an alkene group, preferably norbornene or TCO. According to preferred embodiments, the other click reaction partner comprises a diene, preferably a tetrazine or tetrazole group.

As used herein, the term “covalently linked” means that the polypeptide is attached to the first click reaction partner via at least one covalent linkage, and that the chelant is attached to the second click reaction partner via at least one covalent linkage. The linkage can be direct, i.e. without a linker, or indirect, i.e. via a linker.

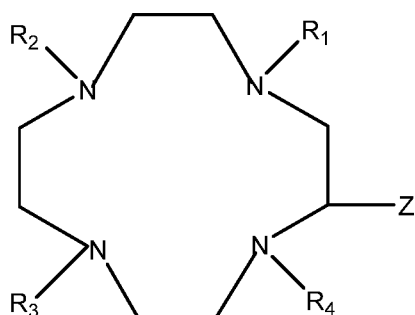
As used herein, the term “linker” refers to a chemical moiety that joins a polypeptide or a chelant to a click reaction partner. Any suitable linker known to those skilled in the art in view of the present disclosure can be used in the invention. The linkers can be, for example, a single covalent bond, a substituted or unsubstituted alkyl, a substituted or unsubstituted heteroalkyl moiety, a polyethylene glycol (PEG) linker, a peptide linker, a sugar-based linker, or a cleavable linker, such as a disulfide linkage or a protease cleavage site such as valine-citrulline-PAB.

As used herein, the term “radiometal ion” or “radioactive metal ion” refers to one or more isotopes of the elements that emit particles and/or photons. Any radioactive metal known to those skilled in the art in view of the present disclosure can be used in the invention. Examples of radioactive metals suitable for use in the invention include, but are not limited to, ^{32}P , ^{47}Sc , ^{62}Cu , ^{64}Cu , ^{67}Cu , ^{67}Ga , ^{68}Ga , ^{77}As , ^{86}Y , ^{89}Zr , ^{89}Sr , ^{90}Y , ^{99}Tc , ^{105}Rh , ^{109}Pd , ^{111}Ag , ^{111}In , ^{117}Sn , ^{131}I , ^{153}Sm , ^{159}Gd , ^{165}Dy , ^{166}Ho , ^{169}Er , ^{177}Lu , ^{186}Re , ^{188}Re , ^{194}Ir , ^{198}Au , ^{199}Au , ^{211}At , ^{212}Pb , ^{212}Bi , ^{213}Bi , ^{223}Ra , ^{225}Ac , ^{227}Th , and ^{255}Fm . As used herein, the term “diagnostic emitter” refers to a radiometal ion that is useful in diagnostic or imaging applications. Examples of diagnostic emitters include, but are not limited to gamma emitters, such as ^{62}Cu , ^{64}Cu , ^{67}Ga , ^{68}Ga , ^{86}Y , ^{89}Zr , and ^{111}In . As used herein, the term “therapeutic emitter” refers to a radiometal ion that is useful

in therapeutic applications. Examples of therapeutic emitters include, but are not limited to, beta or alpha emitters, such as thorium, radium, ^{32}P , ^{47}Sc , ^{67}Cu , ^{77}As , ^{89}Sr , ^{90}Y , ^{99}Tc , ^{105}Rh , ^{109}Pd , ^{111}Ag , ^{131}I , ^{153}Sm , ^{159}Gd , ^{165}Dy , ^{166}Ho , ^{169}Er , ^{177}Lu , ^{186}Re , ^{188}Re , ^{194}Ir , ^{198}Au , ^{199}Au , ^{211}At , ^{212}Pb , ^{212}Bi , ^{213}Bi , ^{223}Ra , ^{225}Ac , ^{255}Fm and ^{227}Th . According to preferred embodiments, the radiometal ion is ^{225}Ac . According to other embodiments, the polypeptide can be labeled with non-metal radiolabels for use in pre-targeting or theranostic applications. Examples of non-metal radiolabels suitable for use in the invention include, but are not limited to, ^{125}I and ^{18}F .

Radiocomplexes described herein comprise a radiometal ion associated with a chelating moiety. According to embodiments of the invention, the chelating moiety comprises a chelant covalently linked to a click reaction partner, and is sometimes referred to herein as a “bifunctional chelator.”

As used herein, the term “chelant” or “chelator” refers to a chemical compound to which a radiometal, such as ^{225}Ac , or metal can be chelated via coordinate bonding. Any chelant known to those skilled in the art in view of the present disclosure can be used in the invention. In an embodiment, the chelant comprises a macrocycle. Examples of chelants comprising a macrocycle suitable for use in the invention include, but are not limited to, deferoxamine (DFO), ethylenediaminetetraacetic acid (EDTA), and diethylenetriaminepentaacetic acid (DTPA). In another embodiment, the chelant comprises an open chain ligand. Examples of chelants comprising an open chain ligand suitable for use in the invention include, but are not limited to, 1,4,7,10-tetraazacyclododecane- $\text{N,N',N'',N'''}\text{-tetraacetic acid (DOTA)}$, 1,4,7,10,13,16-hexaazacyclohexadecane- $\text{N,N',N'',N''',N''''',N''''''}\text{-hexaacetic acid (HEHA)}$, 1,4,7,10,13-pentaazacyclopentadecane- $\text{N,N',N'',N''',N''''}\text{-pentaacetic acid (PEPA)}$, Macropa (Thiele et al., An Eighteen-Membered Macrocyclic Ligand for Actinium-225 Targeted Alpha Therapy. *Angew Chem Int Ed Engl.* 2017 Nov 13;56(46): p. 14712-14717), 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrapropionic acid (DOTPA), 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetrapropionic acid (TETPA), and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid (DOTMP). According to preferred embodiments, the chelant comprises a structure of formula (I):



formula (I),

wherein each of R_1 , R_2 , R_3 and R_4 is independently $CHQCO_2X$, wherein

Q is independently hydrogen, C_1 - C_4 alkyl or (C_1 - C_2 alkyl) phenyl, and

5 X is independently hydrogen, benzyl, C_1 - C_4 alkyl; and

Z is $(CH_2)_nY$, wherein

n is 1-10, and

Y is an electrophilic or nucleophilic moiety covalently linked to the second click reaction partner;

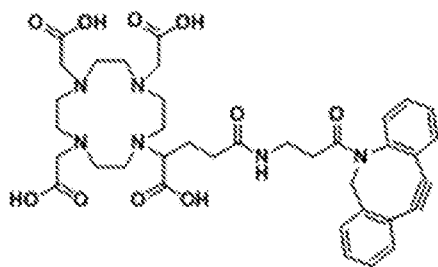
10 alternatively, Z is hydrogen; and

each of R_1 , R_2 , R_3 and R_4 is independently $CHQCO_2X$, wherein

Q is independently hydrogen, C_1 - C_4 alkyl or (C_1 - C_2 alkyl) phenyl, and

X is independently hydrogen, benzyl, C_1 - C_4 alkyl, or an electrophilic or nucleophilic moiety covalently linked to the second click reaction partner.

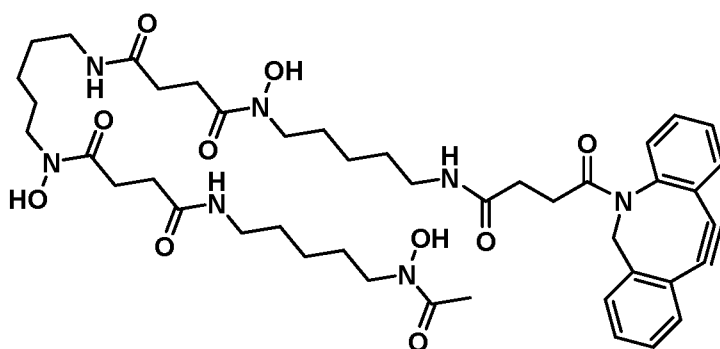
15 According to preferred embodiments, the chelating moiety comprises the structure of formula (II):



formula (II).

According to preferred embodiments, the chelating moiety comprises the structure of

20 formula (III):



formula (III).

In an embodiment, the invention relates to a method of labeling a polypeptide with two or more radiometal ions using a method of the invention. For example, a method of dually labeling

a polypeptide with two radiometal ions comprises:

- a. providing a modified polypeptide comprising the polypeptide covalently linked to a first click reaction partner and a second click reaction partner;
- b. providing a first radiocomplex comprising the first radiometal ion associated with a chelating moiety, wherein the chelating moiety comprises a chelant covalently linked to a third click reaction partner; and
- c. providing a second radiocomplex comprising the second radiometal ion associated with a chelating moiety, wherein the chelating moiety comprises a chelant covalently linked to a fourth click reaction partner; and
- d. contacting the modified polypeptide with the first and second radiocomplexes under a condition to allow the first click reaction partner to react with the third click reaction partner, and the second click reaction partner to react with the fourth click reaction partner, to thereby label the polypeptide with the first and second radiometal ions.

According to preferred embodiments, one of the first and second click reaction partners comprises an alkyne group, and the other of the first and second click reaction partner comprises an azide, and wherein one of the third and fourth click reaction partners comprises an alkene group, and the other of the third and fourth click reaction partner comprises a diene.

According to preferred embodiments, the first or second radiometal ion is a diagnostic emitter, and the other is a therapeutic emitter. According to other preferred embodiments, both the first and second radiometal ions are therapeutic emitters.

Conditions for carrying out click chemistry reactions are known in the art, and any
5 conditions for carrying out click chemistry reactions known to those skilled in the art in view of the present disclosure can be used in the invention. Examples of conditions include, but are not limited to, incubating the modified polypeptide and the radiocomplex at a ratio of 1:1 to 1000:1 at a pH of 4 to 10 and a temperature of 20°C to 70°C.

Products of click radiolabeling methods of the invention can be analyzed using methods
10 known to those skilled in the art in view of the present disclosure. For example, LC/MS analysis can be used to determine the ratio of the chelator to the labeled polypeptide; analytical size-exclusion chromatography can be used to determine the oligomeric state of the polypeptides and polypeptide conjugates; radiochemical yield can be determined by instant thin layer chromatography (e.g. iTLC-SG), and radiochemical purity can be determined by size-exclusion
15 HPLC. Exemplary methods are described herein, e.g., in the Examples below.

Pharmaceutical compositions and methods of treatment

The click radiolabeling method of the invention can be modified into a pre-targeting approach (Kraeber-Bodere, F., et al., A pretargeting system for tumor PET imaging and radioimmunotherapy. *Front Pharmacol*, 2015. 6: p. 54). First, the azido-mAb is dosed, binds to
20 target cells, and is allowed to clear from circulation over time or removed with a clearing agent. Subsequently, the radiocomplex is administered and undergoes the SPAAC reaction with azido-mAbs bound at the target site, while the remaining unbound radiocomplex clears rapidly from circulation (Deal, K.A., et al., Improved in vivo stability of actinium-225 macrocyclic complexes. *J Med Chem*, 1999. 42(15): p. 2988-92). This pre-targeting technique provides a
25 method of enhancing radiometal ion localization at a target site in a subject

Accordingly, in another general aspect, the invention relates to a pharmaceutical composition comprising a radiolabeled polypeptide prepared by a method of the invention and a pharmaceutically acceptable carrier.

As used herein, the term “carrier” refers to any excipient, diluent, filler, salt, buffer, stabilizer, solubilizer, oil, lipid, lipid containing vesicle, microsphere, liposomal encapsulation, or other material well known in the art for use in pharmaceutical formulations. It will be understood that the characteristics of the carrier, excipient or diluent will depend on the route of administration for a particular application. As used herein, the term “pharmaceutically acceptable carrier” refers to a non-toxic material that does not interfere with the effectiveness of a composition according to the invention or the biological activity of a composition according to the invention. According to particular embodiments, in view of the present disclosure, any pharmaceutically acceptable carrier suitable for use in an antibody-based, or a radiocomplex-based pharmaceutical composition can be used in the invention.

According to particular embodiments, the compositions described herein are formulated to be suitable for the intended route of administration to a subject. For example, the compositions described herein can be formulated to be suitable for intravenous, subcutaneous, intramuscular or intratumoral administration.

According to particular embodiments, the modified polypeptide and the radiocomplex can be administered in the same or different compositions.

In another general aspect, the invention relates to a method of treating a neoplastic disease or disorder in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of the invention.

According to particular embodiments, a method of the invention comprises administering a therapeutically effective dose of a pharmaceutical composition of the invention, wherein the composition comprises a radiolabeled polypeptide for targeting cells associated with the neoplastic disease or disorder such that, upon targeting, alpha particles from the ²²⁵Ac and daughters thereof are delivered to the targeted cells and cause a cytotoxic effect thereto, thereby treating the neoplastic disease or disorder.

According to particular embodiments, therapeutically effective amounts of the modified polypeptide and the radiocomplex are administered in different compositions.

As used herein, the term “therapeutically effective amount” refers to an amount of an active ingredient or component that elicits the desired biological or medicinal response in a

subject. A therapeutically effective amount can be determined empirically and in a routine manner, in relation to the stated purpose. For example, in vitro assays can optionally be employed to help identify optimal dosage ranges. Selection of a particular effective dose can be determined (e.g., via clinical trials) by those skilled in the art based upon the consideration of
5 several factors, including the disease to be treated or prevented, the symptoms involved, the patient's body mass, the patient's immune status and other factors known by the skilled artisan. The precise dose to be employed in the formulation will also depend on the route of administration, and the severity of disease, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses can be extrapolated from dose-
10 response curves derived from in vitro or animal model test systems.

As used herein, the terms "treat," "treating," and "treatment" are all intended to refer to an amelioration or reversal of at least one measurable physical parameter related to a disease, disorder, or condition in which administration of a radioactive metal ion would be beneficial, such as a neoplastic disease or disorder, which is not necessarily discernible in the subject, but
15 can be discernible in the subject. The terms "treat," "treating," and "treatment," can also refer to causing regression, preventing the progression, or at least slowing down the progression of the disease, disorder, or condition. In a particular embodiment, "treat," "treating," and "treatment" refer to an alleviation, prevention of the development or onset, or reduction in the duration of one or more symptoms associated with the disease, disorder, or condition in which administration of
20 a radioactive metal ion would be beneficial, such as a neoplastic disease or disorder. In a particular embodiment, "treat," "treating," and "treatment" refer to prevention of the recurrence of the disease, disorder, or condition. In a particular embodiment, "treat," "treating," and "treatment" refer to an increase in the survival of a subject having the disease, disorder, or condition. In a particular embodiment, "treat," "treating," and "treatment" refer to elimination of
25 the disease, disorder, or condition in the subject.

Examples of neoplastic diseases or disorders include, but are not limited to, a disseminated cancer, a solid tumor cancer, a hypertrophy, a coronary disease, or a vascular occlusive disease, a disease or disorder associated with an infected cell, a microbe or a virus, or a disease or disorder associated with an inflammatory cell, such as rheumatoid arthritis (RA).

As used herein, the term “subject” refers to an animal, and preferably a mammal. According to particular embodiments, the subject is a mammal including a non-primate (e.g., a camel, donkey, zebra, cow, pig, horse, goat, sheep, cat, dog, rat, rabbit, guinea pig, marmoset or mouse) or a primate (e.g., a monkey, chimpanzee, or human). In particular embodiments, the
5 subject is a human.

Any dosing schedule of the modified polypeptide and the radiocomplex can be used in view of the present disclosure. In general, when the modified polypeptide and the radiocomplex are administered in different compositions, the radiocomplex can be administered any time after the modified antibody is administered.

10 According to particular embodiments, compositions used in the treatment of a neoplastic disease or disorder can be used in combination with other agents that are effective for treatment of related neoplastic diseases or disorders.

As used herein, the term “in combination,” in the context of the administration of two or more therapies to a subject, refers to the use of more than one therapy. The use of the term “in
15 combination” does not restrict the order in which therapies are administered to a subject. For example, a first therapy (e.g., a composition described herein) can be administered prior to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 16 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (e.g., 5 minutes, 15
20 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 16 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapy to a subject.

In another general aspect, the invention relates to a theranostic agent comprising a radiolabeled antibody prepared by a method of the invention and a pharmaceutically acceptable
25 carrier, wherein the immunological properties of the radiolabeled antibody are preserved.

As used herein, the term “theranostic” refers to the ability to provide either of diagnostic and therapeutic functions. In one embodiment, a theranostic agent provides both diagnostic and therapeutic functions. In another embodiment, a theranostic agent is an active pharmaceutical

agent without diagnostic function. In yet another embodiment, a theranostic agent is an agent useful for diagnosis but having no therapeutic function.

According to preferred embodiments, the radiometal ion is a diagnostic emitter, preferably ^{89}Zr . According to other preferred embodiments, the radiometal ion is a therapeutic emitter, preferably ^{225}Ac . According to preferred embodiments, the theranostic agent is used to provide both diagnostic and therapeutic functions to a subject in need thereof.

Combinations and kits

Provided herein is a combination comprising:

- a. a modified polypeptide comprising a polypeptide covalently linked to a first click reaction partner; and
 - b. a radiocomplex comprising a radiometal ion associated with a chelating moiety, wherein the chelating moiety comprises a chelant covalently linked to a second click reaction partner;
- wherein the combination is to be used for labeling the polypeptide with the radiometal ion.

According to particular embodiments, a combination of the invention is a reaction mixture used to label the polypeptide with the radiometal ion. According to other embodiments, the combination is a pack or kit used to produce a radiolabeled polypeptide, *in vitro* or *in vivo*. Optionally associated with the combination can be a notice or instructions in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. The combinations encompassed herein can be used in the above methods of labeling a polypeptide with a radiometal ion, or of treating a neoplastic disease or disorder in a subject in need thereof.

EMBODIMENTS

The invention provides also the following non-limiting embodiments.

Embodiment 1 is a method of labeling a polypeptide with a radiometal ion, the method comprising:

- a. providing a modified polypeptide comprising the polypeptide covalently linked to a first click reaction partner;
- b. providing a radiocomplex comprising the radiometal ion associated with a chelating moiety, wherein the chelating moiety comprises a chelant covalently linked to a second click reaction partner; and
- c. contacting the modified polypeptide with the radiocomplex under a condition to allow the first click reaction partner to react with the second click reaction partner to thereby label the polypeptide with the radiometal ion.

Embodiment 1a is the method of Embodiment 1, wherein the chelant comprises a macrocycle.

Embodiment 1b is the method of Embodiment 1, wherein the chelant comprises an open chain ligand.

Embodiment 2 is the method of Embodiment 1, wherein one of the first and second click reaction partners comprises an alkyne group, and the other click reaction partner comprises an azide.

Embodiment 3 is the method of Embodiment 2, wherein the first click reaction partner comprises an azide group and the second click reaction partner comprises an alkyne group.

Embodiment 3a is the method of Embodiment 2 or 3, wherein the alkyne group comprises a terminal alkyne.

Embodiment 3b is the method of Embodiment 2 or 3, wherein the alkyne group comprises a cyclic alkynes, preferably a cyclooctyne or cyclooctyne derivative.

Embodiment 3c is the method of Embodiment 3b, wherein the alkyne group comprises bicyclononyne (BCN).

Embodiment 3d is the method of Embodiment 3b, wherein the alkyne group comprises difluorinated cyclooctyne (DIFO).

Embodiment 3e is the method of Embodiment 3b, wherein the alkyne group comprises dibenzocyclooctyne (DIBO).

Embodiment 3f is the method of Embodiment 3b, wherein the alkyne group comprises biarylazacyclooctynone (BARAC).

Embodiment 3g is the method of Embodiment 3b, wherein the alkyne group comprises dibenzoazacyclooctyne (DIBAC).

Embodiment 3h is the method of Embodiment 3b, wherein the alkyne group comprises dimethoxyazacyclooctyne (DIMAC).

5 Embodiment 3i is the method of Embodiment 3b, wherein the alkyne group comprises dibenzoyocyclooctyne (DBCO).

Embodiment 3j is the method of Embodiment 3b, wherein the alkyne group comprises difluorobenzocyclooctyne (DIFBO).

10 Embodiment 3k is the method of Embodiment 3b, wherein the alkyne group comprises monobenzocyclooctyne (MOBO).

Embodiment 3l is the method of Embodiment 3b, wherein the alkyne group comprises tetramethoxy DIBO (TMDIBO).

Embodiment 3m is the method of any one of Embodiments 2 to 3l, wherein the azide group comprises NHS-azide.

15 Embodiment 4 is the method of Embodiment 1, wherein one of the first and second click reaction partners comprises an alkene group, and the other click reaction partner comprises a diene.

Embodiment 4a is the method of Embodiment 4, wherein the diene comprises a tetrazine or tetrazole group.

20 Embodiment 4b is the method of Embodiment 4 or 4a, wherein the alkene group comprises norbornene.

Embodiment 4c is the method of Embodiment 4 or 4a, wherein the alkene group comprises trans-cyclooctene (TCO).

25 Embodiment 5 is the method of any one of Embodiments 1 to 4c, wherein the polypeptide is an antibody or an antigen binding fragment thereof.

Embodiment 6 is the method of Embodiment 5, wherein the antibody is a monoclonal antibody, or an antigen binding fragment thereof.

Embodiment 6a is the method of any one of Embodiments 1 to 6, wherein the modified polypeptide is obtained by randomly conjugating one or more azide groups to the polypeptide.

Embodiment 6b is the method of any one of Embodiments 1 to 6, wherein the modified polypeptide is a modified antibody or antigen binding fragment thereof obtained by site specific incorporation of the first click reaction partner.

Embodiment 6c is the method of Embodiment 6b, wherein the modified antibody or antigen binding fragment thereof is obtained by trimming the antibody or antigen binding fragment thereof with a bacterial endoglycosidase specific for the β -1,4 linkage between the core GlcNAc residue(s) in the Fc-glycosylation site(s) of the antibody to obtain a trimmed antibody or antigen binding fragment thereof, and reacting the trimmed antibody or antigen binding fragment thereof with an azide sugar, preferably UDP-GalNAz azido sugar substrate, in the presence of a sugar transferase, preferably GalT galactosyltransferase.

Embodiment 6d is the method of Embodiment 6b, wherein the modified antibody or antigen binding fragment thereof is obtained by deglycosylating the antibody or antigen binding fragment thereof with an amidase to obtain a deglycosylated antibody or antigen binding fragment thereof, and reacting the deglycosylated antibody or antigen binding fragment thereof with an azido amine, preferably 3-azido propylamine, in the presence of a microbial transglutaminase.

Embodiment 6e is the method of any one of Embodiments 6 to 6d, wherein the antibody is an antibody that binds to human prostate-specific membrane antigen (PSMA) or an antigen binding fragment thereof, preferably the antibody comprises a HC CDR1 sequence of SEQ ID NO: 3, a HC CDR2 sequence of SEQ ID NO: 4, a HC CDR3 sequence of SEQ ID NO: 5, a light chain (LC) CDR1 sequence of SEQ ID NO: 6, a LC CDR2 sequence of SEQ ID NO: 7, and a LC CDR3 sequence of SEQ ID NO: 8.

Embodiment 6f is the method of Embodiment 6e, wherein the antibody comprises a HC sequence of SEQ ID NO: 9 and a LC sequence of SEQ ID NO: 10.

Embodiment 7 is the method of any one of Embodiments 1 to 6d, wherein the radiometal ion is ^{32}P , ^{47}Sc , ^{67}Cu , ^{77}As , ^{89}Sr , ^{90}Y , ^{99}Tc , ^{105}Rh , ^{109}Pd , ^{111}Ag , ^{131}I , ^{153}Sm , ^{159}Gd , ^{165}Dy , ^{166}Ho , ^{169}Er , ^{177}Lu , ^{186}Re , ^{188}Re , ^{194}Ir , ^{198}Au , ^{199}Au , ^{211}At , ^{212}Pb , ^{212}Bi , ^{213}Bi , ^{223}Ra , ^{225}Ac , ^{255}Fm , ^{227}Th , ^{62}Cu , ^{64}Cu , ^{67}Ga , ^{68}Ga , ^{86}Y , ^{89}Zr , or ^{111}In .

Embodiment 7a is the method of any one of Embodiments 1 to 6d, wherein the radiometal ion is ^{225}Ac .

Embodiment 7b is the method of any one of Embodiments 1 to 6d, wherein the radiometal ion is ^{111}In .

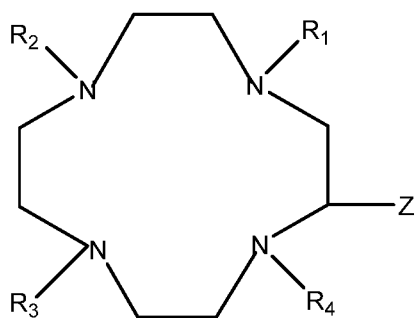
5 Embodiment 7c is the method of any one of Embodiments 1 to 6d, wherein the radiometal ion is ^{89}Zr .

Embodiment 8 is the method of any one of Embodiments 1 to 7c, wherein the chelating moiety is covalently linked to the second click reaction partner via a linker.

10 Embodiment 9 is the method of any one of Embodiments 1 to 8, further comprising reacting an electrophile on a side chain, preferably the amino side chain of a lysine on or introduced to the polypeptide, with a sulfhydryl group covalently linked to the first click reaction partner to obtain the modified polypeptide, preferably NHS-azide.

Embodiment 10 is a method of any one of Embodiments 1 to 9, wherein the modified polypeptide comprises the polypeptide covalently linked, directly or via a linker, to an azide, 15 tetrazine or tetrazole group.

Embodiment 11 is the method of any one of Embodiments 1 to 10, wherein the chelant comprises a macrocycle, preferably a structure of formula (I):



formula (I),

20 wherein each of R_1 , R_2 , R_3 and R_4 is independently CHQCO_2X , wherein

Q is independently hydrogen, $\text{C}_1\text{-C}_4$ alkyl or $(\text{C}_1\text{-C}_2 \text{ alkyl})$ phenyl, and

X is independently hydrogen, benzyl, $\text{C}_1\text{-C}_4$ alkyl; and

Z is $(\text{CH}_2)_n\text{Y}$, wherein

n is 1-10, and

Y is an electrophilic or nucleophilic moiety covalently linked to the second click reaction partner;

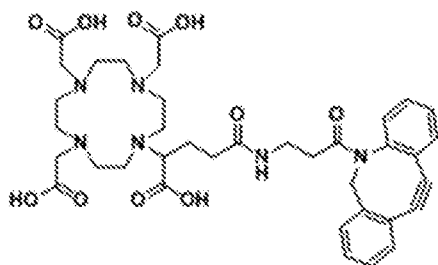
alternatively, Z is hydrogen; and

each of R₁, R₂, R₃ and R₄ is independently CHQCO₂X, wherein

5 Q is independently hydrogen, C₁-C₄ alkyl or (C₁-C₂ alkyl) phenyl, and

X is independently hydrogen, benzyl, C₁-C₄ alkyl, or an electrophilic or nucleophilic moiety covalently linked to the second click reaction partner.

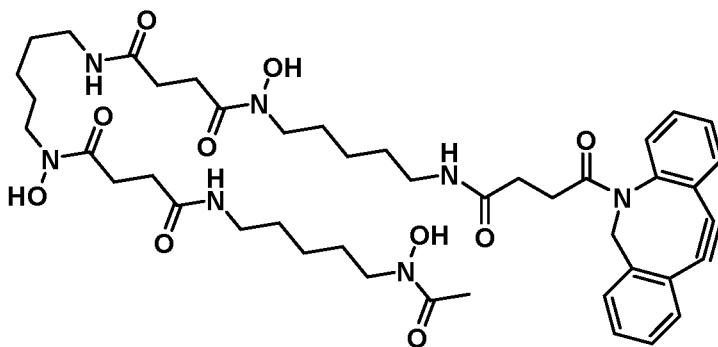
Embodiment 12 is the method of any one of Embodiments 1 to 11, wherein the chelating moiety comprises the structure of formula (II):



10

formula (II).

Embodiment 12a is the method of any one of Embodiments 1 to 11, wherein the chelating moiety comprises a chelant having an open chain ligand, preferably the chelating moiety having a structure of formula (III):



15

formula (III).

Embodiment 12b is the method of any one of Embodiments 1 to 10, wherein the chelating moiety comprises a chelant selected from the group consisting of 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA), deferoxamine (DFO),

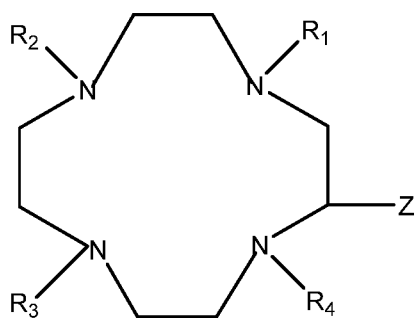
1,4,7,10,13,16-hexaazacyclohexadecane-N,N',N'',N''',N''',N''''-hexaacetic acid (HEHA),
 1,4,7,10,13-pentaazacyclopentadecane-N,N',N'',N''',N''''-pentaacetic acid (PEPA),
 ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA) Macropa
 (Thiele et al., An Eighteen-Membered Macrocyclic Ligand for Actinium-225 Targeted Alpha
 5 Therapy. *Angew Chem Int Ed Engl.* 2017 Nov 13;56(46): p. 14712-14717), 1,4,8,11-
 tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA), 1,4,7,10-tetraazacyclododecane-
 1,4,7,10-tetrapropionic acid (DOTPA), 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetrapropionic
 acid (TETPA), and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid
 (DOTMP).

10 Embodiment 12c is the method of Embodiment 12b, wherein the chelant comprises
 1,4,7,10-tetraazacyclododecane-N,N',N'',N''',N''''-tetraacetic acid (DOTA).

Embodiment 12d is the method of Embodiment 12b, wherein the chelant comprises
 deferoxamine (DFO).

Embodiment 13 is a method of labeling a polypeptide, preferably an antibody or an
 15 antigen binding fragment thereof, with a radiometal ion, preferably ^{225}Ac , ^{111}In or ^{89}Zr , the
 method comprising:

- a. providing a modified polypeptide, preferably a modified antibody or antigen binding
 fragment thereof comprising the polypeptide or antibody or antigen binding fragment
 thereof covalently linked to an azide, tetrazine or tetrazole group;
- 20 b. providing a radiocomplex comprising the radiometal ion, preferably ^{225}Ac , ^{111}In or ^{89}Zr ,
 associated with a chelating moiety, wherein the chelating moiety comprises a chelant
 covalently linked to an alkyne or an alkene group; and
- c. contacting the modified polypeptide or antibody or antigen binding fragment thereof with
 the radiocomplex under a condition to allow the azide, tetrazine or tetrazole group to
 25 react with the alkyne or the alkene group to thereby label the polypeptide or antibody or
 antigen binding fragment thereof with the radiometal ion, preferably ^{225}Ac , ^{111}In or ^{89}Zr ,
 wherein the chelant comprises a structure of formula (I):



formula (I),

wherein each of R₁, R₂, R₃ and R₄ is independently CHQCO₂X, wherein

Q is independently hydrogen, C₁-C₄ alkyl or (C₁-C₂ alkyl) phenyl, and

X is independently hydrogen, benzyl, C₁-C₄ alkyl; and

Z is (CH₂)_nY, wherein

n is 1-10, and

Y is an electrophilic or nucleophilic moiety covalently linked to the alkyne group; alternatively, Z is hydrogen; and

each of R₁, R₂, R₃ and R₄ is independently CHQCO₂X, wherein

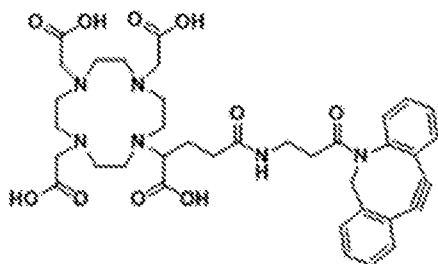
Q is independently hydrogen, C₁-C₄ alkyl or (C₁-C₂ alkyl) phenyl, and

X is independently hydrogen, benzyl, C₁-C₄ alkyl, or an electrophilic or nucleophilic moiety covalently linked to the alkyne group.

Embodiment 13a is the method of Embodiment 13, wherein the chelant comprises

1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA).

Embodiment 13b is the method of Embodiment 13, wherein the chelating moiety comprises the structure of formula (II):



formula (II).

Embodiment 13d is a method of labeling a polypeptide, preferably an antibody or an antigen binding fragment thereof, with a radiometal ion, preferably ^{225}Ac , ^{111}In or ^{89}Zr , the method comprising:

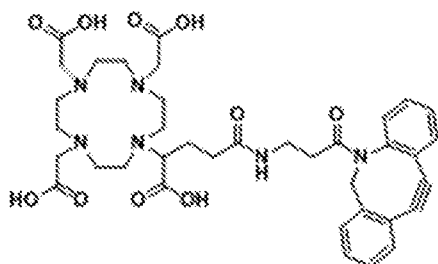
- a. providing a modified polypeptide, preferably a modified antibody or antigen binding fragment thereof comprising the polypeptide or antibody or antigen binding fragment thereof covalently linked to an azide, tetrazine or tetrazole group;
- b. providing a radiocomplex comprising the radiometal ion, preferably ^{225}Ac , ^{111}In or ^{89}Zr , associated with a chelating moiety, wherein the chelating moiety comprises a chelant covalently linked to an alkyne or an alkene group; and
- c. contacting the modified polypeptide or antibody or antigen binding fragment thereof with the radiocomplex under a condition to allow the azide, tetrazine or tetrazole group to react with the alkyne or the alkene group to thereby label the polypeptide or antibody or antigen binding fragment thereof with the radiometal ion, preferably ^{225}Ac , ^{111}In or ^{89}Zr , wherein the chelant comprises an open chain ligand, preferably deferoxamine (DFO).

Embodiment 14 is the method of any one of Embodiments 13 to 13c, wherein the chelant is covalently linked to the alkyne or an alkene group via a linker.

Embodiment 15 is the method of any one of Embodiments 13 to 14, further comprising reacting an electrophile on a side chain, preferably the amino side chain of a lysine on or introduced to the polypeptide, preferably the antibody or antigen binding fragment thereof, with a sulfhydryl group covalently linked to the azide, preferably NHS-azide, to obtain the modified polypeptide or antibody or antigen binding fragment thereof.

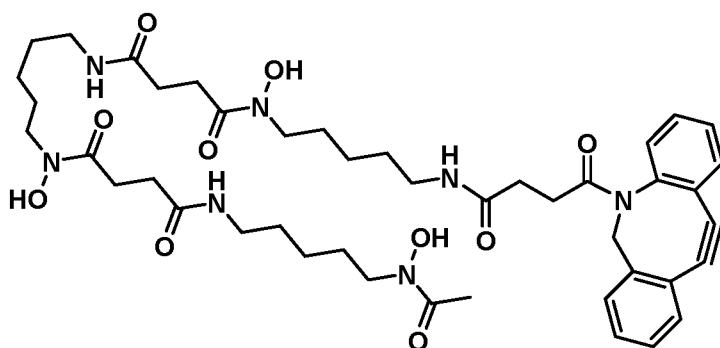
Embodiment 16 is the method of any one of Embodiments 13 to 15, wherein the polypeptide, preferably antibody or antigen binding fragment thereof, is covalently linked to the azide via a linker.

Embodiment 17 is the method of Embodiment 13, wherein the polypeptide is an antibody or antigen binding fragment thereof, the radiometal ion is ^{225}Ac , ^{111}In or ^{89}Zr , and the chelating moiety comprises the structure of formula (II):



formula (II).

Embodiment 17a is the method of Embodiment 13c, wherein the polypeptide is an antibody or antigen binding fragment thereof, the radiometal ion is ^{225}Ac , ^{111}In or ^{89}Zr , and the
 5 chelating moiety comprises the structure of formula (III):



formula (III).

Embodiment 17b is the method of any one of Embodiments 13 to 17a, wherein the polypeptide is an antibody that binds to human prostate-specific membrane antigen (PSMA) or
 10 an antigen binding fragment thereof, preferably the antibody comprises a HC CDR1 sequence of SEQ ID NO: 3, a HC CDR2 sequence of SEQ ID NO: 4, a HC CDR3 sequence of SEQ ID NO: 5, a light chain (LC) CDR1 sequence of SEQ ID NO: 6, a LC CDR2 sequence of SEQ ID NO: 7, and a LC CDR3 sequence of SEQ ID NO: 8.

Embodiment 17c is the method of Embodiment 17b, wherein the antibody comprises a
 15 HC sequence of SEQ ID NO: 9 and a LC sequence of SEQ ID NO: 10.

Embodiment 18 is a method of dually labeling a polypeptide with two radiometal ions, the method comprising:

- a. providing a modified polypeptide comprising the polypeptide covalently linked to a first click reaction partner and a second click reaction partner;

- b. providing a first radiocomplex comprising the first radiometal ion associated with a chelating moiety, wherein the chelating moiety comprises a chelant covalently linked to a third click reaction partner; and
- c. providing a second radiocomplex comprising the second radiometal ion associated with a chelating moiety, wherein the chelating moiety comprises a chelant covalently linked to a fourth click reaction partner; and
- d. contacting the modified polypeptide with the first and second radiocomplexes under a condition to allow the first click reaction partner to react with the third click reaction partner, and the second click reaction partner to react with the fourth click reaction partner, to thereby label the polypeptide with the first and second radiometal ions.

Embodiment 19 is the method of Embodiment 18, wherein one of the first and second click reaction partners comprises an alkyne group, and the other of the first and second click reaction partner comprises an azide, and wherein one of the third and fourth click reaction partners comprises an alkene group, and the other of the third and fourth click reaction partner comprises a diene.

Embodiment 20 is the method of Embodiment 18 or 19, wherein the first or second radiometal ion is a diagnostic emitter, and the other is a therapeutic emitter.

Embodiment 21 is the method of Embodiment 18 or 19, wherein both the first and second radiometal ions are therapeutic emitters.

Embodiment 21a is the method of Embodiment 20 or 21, wherein the diagnostic emitter is ^{62}Cu , ^{64}Cu , ^{67}Ga , ^{68}Ga , ^{86}Y , ^{89}Zr , or ^{111}In .

Embodiment 21b is the method of any one of Embodiments 20 to 21a, wherein the therapeutic emitter is ^{32}P , ^{47}Sc , ^{67}Cu , ^{77}As , ^{89}Sr , ^{90}Y , ^{99}Tc , ^{105}Rh , ^{109}Pd , ^{111}Ag , ^{131}I , ^{153}Sm , ^{159}Gd , ^{165}Dy , ^{166}Ho , ^{169}Er , ^{177}Lu , ^{186}Re , ^{188}Re , ^{194}Ir , ^{198}Au , ^{199}Au , ^{211}At , ^{212}Pb , ^{212}Bi , ^{213}Bi , ^{223}Ra , ^{225}Ac , ^{255}Fm or ^{227}Th .

Embodiment 22 is a pharmaceutical composition comprising a radiolabeled polypeptide prepared by a method of any one of Embodiments 1 to 21b and a pharmaceutically acceptable carrier.

Embodiment 23 is a method of treating or diagnosing a disease or disorder, particularly a neoplastic disease or disorder, in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of Embodiment 22.

Embodiment 24 is the method of Embodiment 23, wherein the pharmaceutical
5 composition comprises two compositions to be administered sequentially, the first comprising the modified polypeptide, and the second comprising the radiocomplex or radiocomplexes.

Embodiment 25 is a theranostic agent comprising a radiolabeled antibody prepared by a method of any one of Embodiments 1 to 21b and a pharmaceutically acceptable carrier, wherein the immunological properties of the radiolabeled antibody are preserved.

10 Embodiment 26 is the theranostic agent of Embodiment 25, wherein the radiometal ion is a diagnostic emitter, preferably ^{89}Zr .

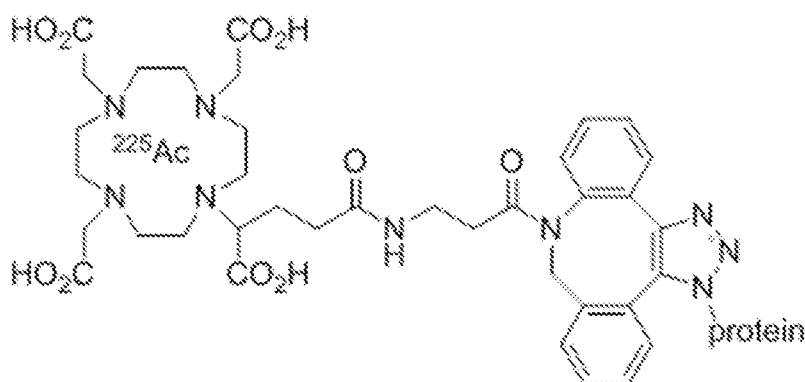
Embodiment 27 is the theranostic agent of Embodiment 25, wherein the radiometal ion is a therapeutic emitter, preferably ^{225}Ac .

15 Embodiment 27a is the theranostic agent of Embodiment 25, wherein the radiometal ion is ^{111}In .

Embodiment 27b is the theranostic agent of any one of Embodiments 25 to 27a, wherein the polypeptide is antibody that binds to human prostate-specific membrane antigen (PSMA) or an antigen binding fragment thereof, preferably the antibody comprises a HC CDR1 sequence of SEQ ID NO: 3, a HC CDR2 sequence of SEQ ID NO: 4, a HC CDR3 sequence of SEQ ID NO:
20 5, a light chain (LC) CDR1 sequence of SEQ ID NO: 6, a LC CDR2 sequence of SEQ ID NO: 7, and a LC CDR3 sequence of SEQ ID NO: 8.

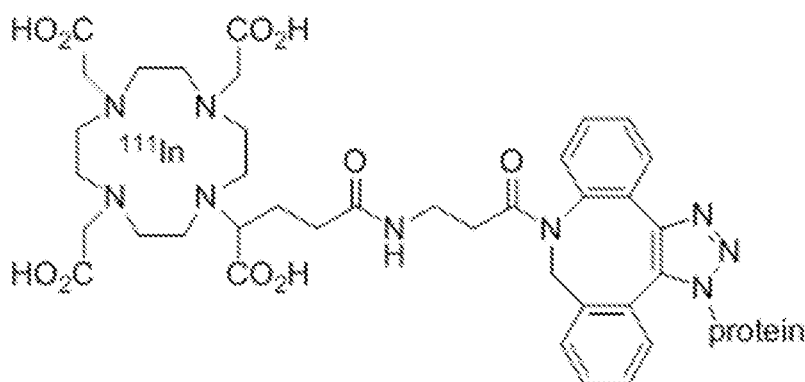
Embodiment 27c is the theranostic agent of Embodiment 27b, wherein the antibody comprises a HC sequence of SEQ ID NO: 9 and a LC sequence of SEQ ID NO: 10.

25 Embodiment 27d is the theranostic agent of any one of Embodiments 25 to 27c, wherein the radiolabeled antibody has the formula of formula (IV):



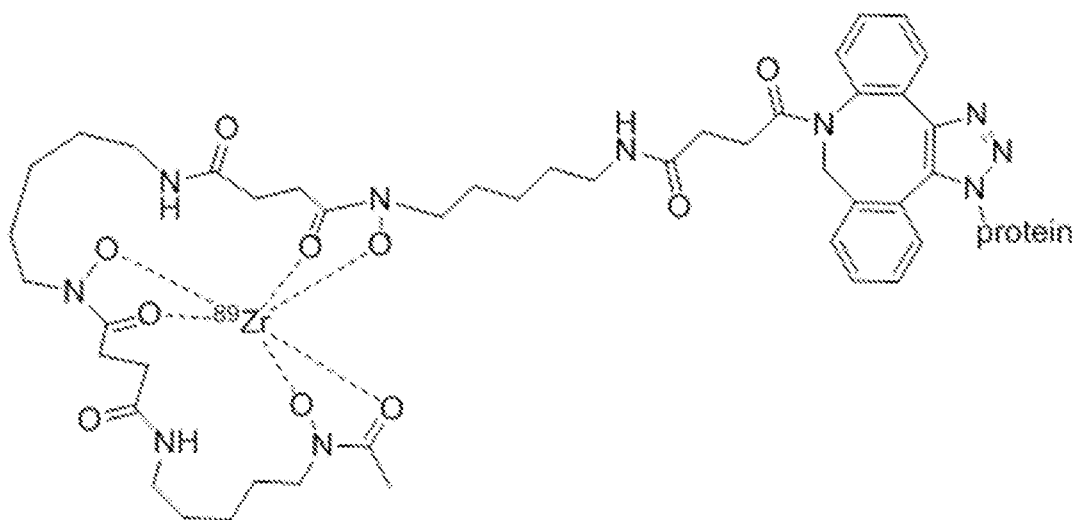
formula (IV) (DOTA-Ac-DBCO-protein), alternatively, the ^{225}Ac is substituted with another radiometal ion, such as ^{32}P , ^{47}Sc , ^{67}Cu , ^{77}As , ^{89}Sr , ^{90}Y , ^{99}Tc , ^{105}Rh , ^{109}Pd , ^{111}Ag , ^{131}I , ^{153}Sm , ^{159}Gd , ^{165}Dy , ^{166}Ho , ^{169}Er , ^{177}Lu , ^{186}Re , ^{188}Re , ^{194}Ir , ^{198}Au , ^{199}Au , ^{211}At , ^{212}Pb , ^{212}Bi , ^{213}Bi , ^{223}Ra , ^{255}Fm , ^{227}Th , ^{62}Cu , ^{64}Cu , ^{67}Ga , ^{68}Ga , ^{86}Y , ^{89}Zr , or ^{111}In .

Embodiment 27e is the theranostic agent of any one of Embodiments 25 to 27c, wherein the radiolabeled antibody has the formula of formula (V):



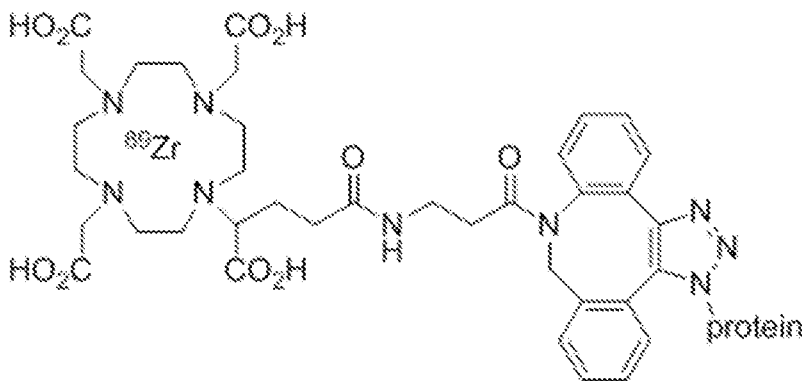
formula (V) (DOTA-In-DBCO-protein).

Embodiment 27f is the theranostic agent of any one of Embodiments 25 to 27c, wherein the radiolabeled antibody has the formula of formula (VI):



formula (VI) (DFO-Zr-DBCO-protein), alternatively, the ^{89}Zr is substituted with another radiometal ion, such as ^{32}P , ^{47}Sc , ^{67}Cu , ^{77}As , ^{89}Sr , ^{90}Y , ^{99}Tc , ^{105}Rh , ^{109}Pd , ^{111}Ag , ^{131}I , ^{153}Sm , ^{159}Gd , ^{165}Dy , ^{166}Ho , ^{169}Er , ^{177}Lu , ^{186}Re , ^{188}Re , ^{194}Ir , ^{198}Au , ^{199}Au , ^{211}At , ^{212}Pb , ^{212}Bi , ^{213}Bi , ^{223}Ra , ^{225}Ac , ^{255}Fm , ^{227}Th , ^{62}Cu , ^{64}Cu , ^{67}Ga , ^{68}Ga , ^{86}Y , or ^{111}In .

Embodiment 27g is the theranostic agent of any one of Embodiments 25 to 27c, wherein the radiolabeled antibody has the formula of formula (VII):



formula (VII) (DOTA-Zr-DBCO-protein).

Embodiment 27h is the theranostic agent of any one of Embodiments 25 to 27g, wherein the radiolabeled antibody has a chelator:antibody ratio (CAR) of less than 3.

Embodiment 27i is the theranostic agent of any one of Embodiments 25 to 27h, wherein the radiolabeled antibody has a chelator:antibody ratio (CAR) of 2.

Embodiment 28 is a combination, preferably a kit, comprising:

- a. a modified polypeptide comprising a polypeptide covalently linked to a first click reaction partner; and
- b. a radiocomplex comprising a radiometal ion associated with a chelating moiety, wherein the chelating moiety comprises a chelant covalently linked to a second click reaction partner;
- 5 wherein the combination is to be used for labeling the polypeptide with the radiometal ion.

Embodiment 28a is the combination or kit of Embodiment 28, wherein the chelant comprises a macrocycle.

- 10 Embodiment 28b is the combination or kit of Embodiment 28, wherein the chelant comprises an open chain ligand.

Embodiment 29 is the combination or kit of Embodiment 28, which is to be used for labeling the polypeptide with the radiometal ion via a reaction between the first and second click reaction partners *in vitro*.

- 15 Embodiment 30 is the combination or kit of Embodiment 28, which is to be used for labeling the polypeptide with the radiometal ion via a reaction between the first and second click reaction partners *in vivo*.

Embodiment 31 is a composition comprising a modified polypeptide comprising a polypeptide covalently linked to a first click reaction partner.

- 20 Embodiment 32 is a composition comprising a radiocomplex comprising a radiometal ion associated with a chelating moiety, wherein the chelating moiety comprises a chelant covalently linked to a second click reaction partner.

Embodiment 32a is the composition of Embodiment 32, wherein the chelant comprises a macrocycle.

- 25 Embodiment 32b is the composition of Embodiment 32, wherein the chelant comprises an open chain ligand.

Embodiment 33 is the combination or kit of any one of Embodiments 28 to 30 or the composition of Embodiment 31 or 32, wherein the polypeptide is an antibody or an antigen binding fragment thereof.

Embodiment 33a is the combination or kit or the composition of Embodiment 33,
5 wherein the antibody is capable of binding to human prostate-specific membrane antigen (PSMA) or an antigen binding fragment thereof, preferably the antibody comprises a HC CDR1 sequence of SEQ ID NO: 3, a HC CDR2 sequence of SEQ ID NO: 4, a HC CDR3 sequence of SEQ ID NO: 5, a light chain (LC) CDR1 sequence of SEQ ID NO: 6, a LC CDR2 sequence of SEQ ID NO: 7, and a LC CDR3 sequence of SEQ ID NO: 8.

10 Embodiment 33b is the combination or kit or the composition of Embodiment 33a, wherein the antibody comprises a HC sequence of SEQ ID NO: 9 and a LC sequence of SEQ ID NO: 10.

Embodiment 33c is the combination or kit or the composition of Embodiment 33a or 33b, wherein the antibody or antigen binding fragment thereof is covalently linked to an azide group.

15 Embodiment 33d is the combination or kit or the composition of Embodiment 33c, wherein the antibody or antigen binding fragment thereof is covalently linked to the azide group randomly.

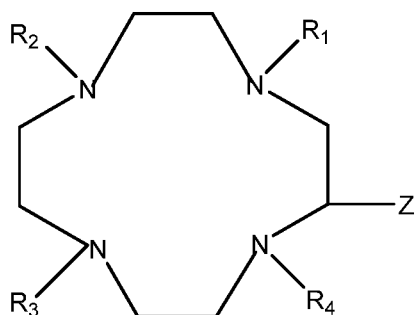
Embodiment 33e is the combination or kit or the composition of Embodiment 33c, wherein the antibody or antigen binding fragment thereof is covalently linked to the azide group
20 site specifically.

Embodiment 33f is the combination or kit or the composition of Embodiment 33e, wherein the antibody or antigen binding fragment thereof is covalently linked to the azide group via a method comprising: trimming the antibody or antigen binding fragment thereof with a bacterial endoglycosidase specific for the β -1,4 linkage between the core GlcNac residue(s) in
25 the Fc-glycosylation site(s) of the antibody to obtain a trimmed antibody or antigen binding fragment thereof, and reacting the trimmed antibody or antigen binding fragment thereof with an azide sugar, preferably UDP-GalNaz azido sugar substrate, in the presence of a sugar transferase, preferably GalT galactosyltransferase.

Embodiment 33g is the combination or kit or the composition of Embodiment 33e, wherein the modified antibody or antigen binding fragment thereof is obtained by a method comprising deglycosylating the antibody or antigen binding fragment thereof with an amidase to obtain a deglycosylated antibody or antigen binding fragment thereof, and reacting the
 5 deglycosylated antibody or antigen binding fragment thereof with an azido amine, preferably 3-azido propylamine, in the presence of a microbial transglutaminase.

Embodiment 34 is the combination, kit or composition of any one of Embodiments 33 to 33g, wherein the radiometal ion comprises ^{225}Ac , ^{111}In or ^{89}Zr .

Embodiment 35 is the combination, kit or composition of Embodiment 34, wherein the
 10 chelant comprises a macrocycle, preferably a structure of formula (I):



formula (I),

wherein each of R_1 , R_2 , R_3 and R_4 is independently CHQCO_2X , wherein

Q is independently hydrogen, C_1 - C_4 alkyl or (C_1 - C_2 alkyl) phenyl, and

15 X is independently hydrogen, benzyl, C_1 - C_4 alkyl; and

Z is $(\text{CH}_2)_n\text{Y}$, wherein

n is 1-10, and

Y is an electrophilic or nucleophilic moiety covalently linked to the alkyne group;

alternatively, Z is hydrogen; and

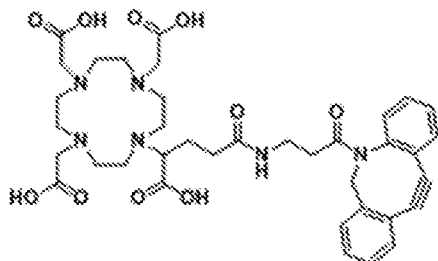
20 each of R_1 , R_2 , R_3 and R_4 is independently CHQCO_2X , wherein

Q is independently hydrogen, C_1 - C_4 alkyl or (C_1 - C_2 alkyl) phenyl, and

X is independently hydrogen, benzyl, C_1 - C_4 alkyl, or an electrophilic or nucleophilic moiety covalently linked to the alkyne group.

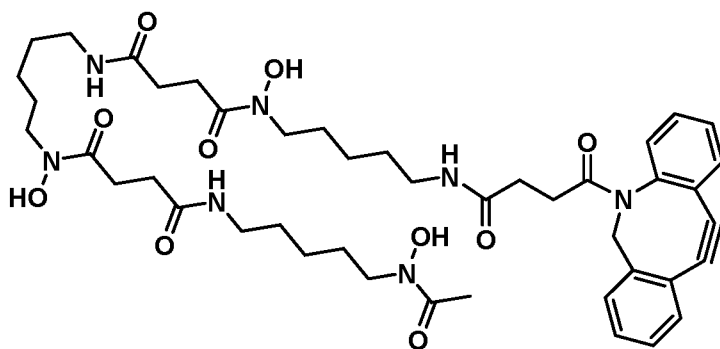
Embodiment 36 is the combination, kit or composition of Embodiment 35, wherein the electrophilic or nucleophilic moiety is covalently linked to the alkyne group via a linker.

Embodiment 37 is the combination, kit or composition of Embodiment 35 or 36, wherein the chelating moiety comprises a structure of formula (II):



formula (II)

Embodiment 37a is the combination, kit or composition of Embodiment 34, wherein the chelating moiety comprises a chelant having an open chain ligand, preferably the chelating moiety comprises a structure of formula (III):



formula (III).

Embodiment 38 is the combination, kit or composition of any one of Embodiments 34 to 37, wherein the polypeptide is covalently linked to the azide via a linker.

Embodiment 39 is a method of treating or diagnosing a disease or disorder, particularly a neoplastic disease or disorder, in a subject in need thereof, comprising administering to the subject the theranostic agent of any one of Embodiments 25-27d, or the combination of any one of Embodiments 28 and 33 to 38.

Embodiment 40 is a method of treating or diagnosing a disease or disorder, particularly a neoplastic disease or disorder, in a subject in need thereof, comprising administering to the

subject the composition of Embodiment 31 and the composition of Embodiment 32, preferably the polypeptide is an antibody.

EXAMPLES

The following examples of the invention are to further illustrate the nature of the invention. It should be understood that the following examples do not limit the invention and that the scope of the invention is to be determined by the appended claims.

Example 1: Random conjugation of azide/handle to an antibody

Monoclonal Antibodies (mAbs): A human IgG4 antibody that binds to human prostate-specific membrane antigen (PSMA), referred to herein as “anti-PSMA mAb” with designation “PSMB127”, has a heavy chain (HC) CDR1 sequence of SEQ ID NO: 3, a HC CDR2 sequence of SEQ ID NO: 4, a HC CDR3 sequence of SEQ ID NO: 5, a light chain (LC) CDR1 sequence of SEQ ID NO: 6, a LC CDR2 sequence of SEQ ID NO: 7, and a LC CDR3 sequence of SEQ ID NO: 8, and has a HC sequence of SEQ ID NO: 9 and a LC sequence of SEQ ID NO: 10. Anti-PSMA mAb was expressed and purified using standard chromatography methods.

A human IgG4 S228P/F234A/L235A (IgG4-PAA) antibody isotype control for the anti-PSMA mAb, referred to herein as “control mAb”, has a HC sequence of SEQ ID NO: 1 and a LC sequence of SEQ ID NO: 2. The commercial antibodies trastuzumab (Herceptin), cetuximab (Erbix), pertuzumab (Perjeta), and panitumumab (Vectibix) were purchased from Roche, Lilly, Roche, and Amgen respectively. Mouse anti-human Her2 mAb was obtained from BioXCell (catalog #BE0277). Trastuzumab, pertuzumab, and anti-human Her2 mAb bind to human Her2. Cetuximab and panitumumab bind to human EGFR.

Conjugation: A stock solution of the antibody (1-10 mg/mL) in 10 mM sodium acetate pH 5.2, phosphate-buffered saline pH 7, or other compatible buffer was mixed with 20% (v/v) of 1 M sodium carbonate buffer pH 9 to a final pH of ~9. NHS-PEG4-azide (Thermo catalog #26130) was dissolved in DMSO to a final concentration of 100 mM, and 0.2% (v/v) of the stock was added to produce a molar excess of ~3-10 relative to the mAb. The reaction was incubated at

22°C for 10 minutes followed by quenching with the addition of 1 M Tris pH 7.5 to a final concentration of 50 mM Tris.

Purification: The azide-mAb conjugate was purified and exchanged into a compatible buffer (PBS; 20 mM HEPES 150 mM, NaCl pH 7.5; or 10 mM sodium acetate pH 5.2) using a method such as Zeba desalting columns with 7K MW cutoff (Thermo), dialysis; standard protein A affinity chromatography; or another compatible method. After purification, the conjugate was concentrated to 10-20 mg/mL using Amicon concentrators with 50K MW cutoff (Millipore).

LC/MS analysis: The chelator:antibody ratio (CAR) was determined by LC/MS analysis with an Agilent G6224 MS-TOF instrument equipped with an Agilent PLRP-S column (300-Ångstrom, 2.1x150 mm; catalog #PL1912-3301) (Table 1). The mass spectrum was deconvoluted using the maximum entropy algorithm over the m/z range 2000-3200 for the masses 140-170 kDa.

Analytical size-exclusion chromatography (SEC): Analytical SEC was performed to determine the oligomeric state of the antibodies and antibody conjugates and ensure that the conjugation process had not led to aggregation. An Agilent 1200 series HPLC equipped with a Tosoh TSKgel G3000SWxl (Tosoh Bioscience #08541) 7.8mm x 30cm column was used; mobile phase 1X PBS; flow rate 0.8 ml/ml; injection volume 15 µL; protein concentration 0.1-2 mg/mL.

Table 1: Conjugation efficiency

Antibody	Isotype	CAR
anti-PSMA mAb	Human IgG4	2.4
control mAb	Human IgG4	2.0
Trastuzumab	Humanized IgG1	2.5
Cetuximab	Chimeric human/mouse IgG1	3.6
Pertuzumab	Humanized IgG1	2.3
Panitumumab	Human IgG2	3.0
Anti-human Her2	Mouse IgG2a	3.1

Example 2: Random conjugation of azide/handle to non-antibody polypeptides

Non-antibody polypeptides: Transferrin, human holo-Transferrin was purchased from R&D Systems (catalog #2914-HT) and dissolved in water to 10 mg/mL. EGF, human epidermal growth factor (EGF) was purchased from Sino Biological (catalog #10605-HNAE).

5 **Conjugation:** A stock solution of the polypeptides (1-10 mg/mL) in 10 mM sodium acetate pH 5.2, phosphate-buffered saline pH 7, or other compatible buffer, was mixed with 20% (v/v) of 1 M sodium carbonate buffer pH 9 to a final pH of ~9. NHS-PEG4-azide (Thermo catalog #26130) was dissolved in DMSO to a final concentration of 100 mM, and 0.2% (v/v) of the stock was added to produce a molar excess of ~3-10 relative to the protein. The reaction was
10 incubated at 22°C for 10 minutes followed by quenching with 1 M Tris pH 7.5 to a final concentration of 50 mM. The conjugation efficiency is shown in Table 2.

Table 2: Conjugation Efficiency

Protein	Chelator:protein ratio
Transferrin	3.6
EGF	1.1

15 **Example 3: Site-specific incorporation of azido sugars into antibody glycans**

Antibody glycans were trimmed with GlycINATOR (Genovis), a bacterial endoglycosidase specific for the β -1,4 linkage between the core GlcNAc residues in the Fc-glycosylation site(s) leaving the inner most GlcNAc intact on the Fc, which can then be used for site-specific incorporation of azido sugars. More specifically, immobilized GlycINATOR on
20 agarose beads packed into a column (Genovis) was equilibrated in Tris-buffered saline pH 7.4 (TBS). 1 mL of mAb at 5-10 mg/mL was added to the resin and incubated on a rocker for 1 hour at RT. mAb was eluted by spinning at 100x g for 1 minute. The column was eluted 3 more times with 0.5 mL TBS. The elutions containing trimmed mAb were pooled, and the supplied buffer additive (Genovis) was added along with UDP-GalNaz azido sugar substrate and GalT
25 galactosyltransferase enzyme. The reaction was incubated overnight at 30°C. The final azido

mAb was purified using a mAb Select column (GE) on an AKTA Avant instrument. Azide modification was confirmed by LC-MS with the CAR determined to be exactly 2.

Example 4: Site-specific incorporation of 3-azido propylamine with microbial transglutaminase (MTG)

Azido groups were installed site-specifically on the antibody at positions Gln295 essentially as described (Dennler et al. Transglutaminase-based chemo-enzymatic conjugation approach yields homogeneous antibody-drug conjugates. *Bioconj Chem* 2014 Mar 19;25(3): p. 569-78). Anti-PSMA mAb was deglycosylated with Rapid PNGase F (New England Biolabs), an amidase which cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and complex oligosaccharides and allows for complete deglycosylation and release of all N-glycans, including N-glycans from both conserved (e.g., Fc Asn297) and non-conserved (e.g., Fab N-glycans) glycosylation sites. 10 mL of antibody at 1 mg/mL in sodium acetate buffer pH 5.2 was incubated with 5 μ L of PNGase F overnight at 37°C, and deglycosylation was confirmed by LC-MS. PNGase F was removed by 4 cycles of concentration and dilution with an Amicon device (50 kDa cutoff). For conjugation of 3-azido propylamine (3-APA; Click Chemistry Tools), the deglycosylated mAb (0.5-1 mg/mL) was brought to pH 7-7.5 by addition of 2% (v/v) of 0.5 M HEPES pH 7.5. 100 equivalents of 3-APA were added along with 5-10% w/v of Activa TI transglutaminase (Ajinomoto), an MTG. The reaction was incubated at 37°C for 1-4 hours followed by purification of the azide-modified mAb on a mAbSelect Sure column using standard chromatographic methods. The conjugate was characterized by LC-MS and the CAR was determined to be 2.

Example 5: Chelation of radiometal to bifunctional chelator (BFC)

Synthesis of ^{225}Ac -DOTA-GA-DBCO: $^{225}\text{Ac}(\text{NO}_3)_3$ was purchased from Oak Ridge National Laboratory. 1,4,7,10-tetraazacyclododececan-1-(glutaric acid)-4,7,10-triacetic acid-(3-amino-propanoic acid)dibenzocyclo-octyne (DOTA-GA-DBCO) was custom synthesized. The synthesis was based on Bernhard et al. *Chem. Eur. J.* **2012**, *18*, 7834-7841. DBCO-amine (3-

Amino-1-[(5-aza-3,4:7,8-dibenzocyclooct-1-yn-5-yl)-1-propanone, Sigma) was reacted with the DOTA-GA anhydride and the product was purified by reverse-phase HPLC.

Quantification of Actinium-225 was achieved using a Capintec CRC-55TW dose calibrator whereby $^{225}\text{Ac}(\text{NO}_3)_3$ dissolved in 0.1 N HCl to make a 10 mCi/mL solution. To a solution of tetramethylammonium acetate (1 M solution, 7.5 μL , 7.5 μmol), DOTA-GA-DBCO (1 mg/mL in water, 2.5 μL , 3.4 nmol) and NaOH (0.1 N, 2.5 μL , 0.25 μmol) in a plastic vial was added to $^{225}\text{Ac}(\text{NO}_3)_3$ (10 mCi/mL in 0.1 N HCl, 5 μL , 50 μCi , 0.0038 nmol). The pH of the mixture was observed to be ~ 6.5 by pH paper. The vial was placed on a shake block at 80°C and 290 rpm for 30 min., and the vial was allowed to cool to room temperature.

Synthesis of ^{111}In -DOTA-GA-DBCO: $^{111}\text{InCl}_3$ in 0.05 M HCl was purchased from GE Healthcare. To a solution of tetramethylammonium acetate (1 M solution, 7.5 μL , 7.5 μmol), DOTA-GA-DBCO (1 mg/mL in water, 2.5 μL , 3.4 nmol) and HCl (0.1 N, 5 μL) in a plastic vial was added $^{111}\text{InCl}_3$ in 0.05 N HCl (5 μL , 104.3 μCi measured in a Capintec CRC-55TW dose calibrator, 0.0022 nmol). The pH of the mixture was observed to be ~ 5.5 by pH paper. The vial was placed on a shake block at 60°C and 290 rpm for 30 min., and the vial was allowed to cool to room temperature.

Synthesis of ^{89}Zr -DFO-DBCO: ^{89}Zr oxalate was purchased from 3D Imaging. DFO-DBCO was purchased from Macrocyclics (Plano, TX catalog #B-773), dissolved in DMSO to 0.5 mg/mL, and diluted in water to 25 $\mu\text{g/mL}$.

2 mCi of Zr-89 was transferred to a metal-free micro-centrifuge tube, and 1 M oxalic acid was added to reach a total volume of 80 μL . 12 μL of 2 M potassium carbonate was added in 2 μL increments, and the mixture stirred with a pipet tip until bubbling stopped. 120 μL of 1 M HEPES was then added followed by 300 μL of water. The pH of the solution was tested, and additional 2 M potassium carbonate was added to raise the pH to 6-6.5 if necessary. 136 μL of the DFO-DBCO stock (3.4 μg) was added, and the reaction was incubated for 1 hour at RT. The chelate was analyzed by spotting 0.5 μL of the reaction on a TLC Green strip (Biodex) and eluting with 20% NaCl. After elution, the strip was scanned with a PerkinElmer Cyclone Plus phosphor imager to ensure that the majority of the Zr-89 remained at the baseline, indicating chelation by DFO-DBCO.

Synthesis of ^{89}Zr -DOTA-GA-DBCO: To a Waters Sep-pak Light QMA strong anion exchange cartridge (acrylic acid/acrylamide copolymer on Diol silica, Surface functionality: $\text{C}(\text{O})\text{NH}(\text{CH}_2)_3\text{N}(\text{CH}_3)_3^+\text{Cl}^-$, 300 Å pore size, 37–55 µm particle size, 230 µeq/gram ion exchange capacity), MeCN (6 mL) was added followed by 0.9% saline (10 mL) then water (10 mL). A solution of $^{89}\text{Zr}(\text{ox})_2$ in 1.0 M oxalic acid (2 µL, 290 µCi) was added to the pre-conditioned cartridge. The cartridge was in turn washed with de-ionized water (20 ml) to remove excess oxalic acid followed by elution of $^{89}\text{ZrCl}_4$ off the column with 1.0 M HCl(aq.) (100 µL each) to afford a total volume of 400 µL with recovery of 248 µCi (86%), the majority of activity being in fraction 3. The combined aliquots were then evaporated to dryness.

10 10 µL of DOTA-GA-DBCO (1.0 mg/mL in metal free water, 10 µg, 13.6 nmol) was added to the $^{89}\text{ZrCl}_4$ (268 uCi, 50 µL). The solution of DOTA-GA-DBCO/ ^{89}Zr was diluted in 150 µL of 1.0 M HEPES. The pH of the mixture was adjusted to pH 7.5 (Pandya et al., Zirconium tetraazamacrocyclic complexes display extraordinary stability and provide a new strategy for zirconium-89-based radiopharmaceutical development. *Chem Sci.* 2017 Mar 1;8(3): p. 2309-2314). The solution was then incubated at 90°C for 60 min. The yield of ^{89}Zr -DOTA-GA-DBCO complex was determined to be 98% by SPC25 column (Sigma Aldrich part number SPC25120-50G) eluted with 1% NH_4OH solution. Un-chelated ^{89}Zr stays on the column, while ^{89}Zr -DOTA-GA-DBCO complex is eluted out.

20 **Example 6: Synthesis of click labeled radioconjugates of anti-PSMA mAb**

See Figs. 1 and 2 for schematics of schematics of radiolabeling antibodies according to methods of the invention.

Synthesis of anti-PSMA mAb-dibenzo-[1,2,3]-triazoloazocine-GA-DOTA-225Ac (site specific, CAR = 2): Random or site-specific azide-modified antibody (site-specific, CAR=2 or random, average CAR between 1 and 4) in PBS or other compatible buffer (10-20 mg/mL) was added to the solution of ^{225}Ac -DOTA-GA-DBCO produced as described. The final pH of the mixture was ~6.5 by pH paper. The reaction solution was gently agitated and allowed to stand still at room temperature for 3h before purification with a PD-10 column (GE Healthcare) pre-conditioned with 15 mL of NaOAc buffer, 10 mM, pH 6-6.5 or another compatible buffer. The

reaction mixture was pipetted into the reservoir of the pre-conditioned PD-10 column, and the eluate was collected in plastic tubes. The reaction vial was washed with NaOAc buffer (0.2 mL x 3), the washings were pipetted into the reservoir of the PD-10 column, and the eluate was collected. NaOAc buffer was continuously applied into the reservoir of the PD-10 column, and the eluate was collected in plastic tubes with each tube collecting ~1 mL of the eluate, until a total of 10 mL of eluate was collected.

The purity of each fraction collected was assessed by iTLC-SG (Agilent) using citrate-H₂O-MeOH solution as the mobile phase. Pure fraction(s) were combined to afford final product in 10mM NaOAc buffer. The product solution was analyzed by HPLC for chemical and radiochemical purity. The antibody concentration in the product solution was determined by UV absorption using a standard curve. The activity of the product solution was in turn quantified using a Capintec CRC-55TW dose calibrator.

Quality control of Ac-225 chelation: A chelation challenge was employed using diethylenetriaminepentaacetic acid (DTPA) as a quality control for a purified product. A 10 mM aqueous solution of Na₅DTPA was added to a sample solution containing ²²⁵Ac labeled mAb until the [DTPA]/[mAb] = 500 - 1,000. A 50 mM aqueous solution of Na₅DTPA was added to an aliquot of the purified product in solution containing ²²⁵Ac labeled mAb until the [DTPA]/[mAb] = 50,000 - 100,000. The two mixtures were placed on a shake block at room temperature and 290 rpm for 30 min. The mixture was spotted on iTLC-SG and developed with citrate-H₂O-MeOH solution as the mobile phase. Under these conditions, free ²²⁵Ac migrates to the solvent front and the bound ²²⁵Ac-mAb stays at the baseline.

Synthesis of anti-PSMA mAb-dibenzo-[1,2,3]-triazoloazocine-GA-DOTA-¹¹¹In:

Random or site-specific azide-modified anti-PSMA mAb in 10 mM NaOAc (site-specific, CAR=2 or random, average CAR between 1 and 4) was added to the solution of ¹¹¹In-DOTA-GA-DBCO produced as described. The reaction solution was gently agitated and allowed to stand still at room temperature for 2h before passing through a PD-10 column. The PD-10 column was pre-conditioned by passing 15 mL of NaOAc buffer, 10 mM, pH 6-6.5, through the column, and the washings were discarded. The reaction mixture was then pipetted into the reservoir of the pre-conditioned PD-10 column, and the eluate was collected in plastic tubes. The

reaction vial was washed with NaOAc buffer (0.2 mL x 3), the washings were pipetted into the reservoir of the PD-10 column, and the eluate was collected. NaOAc buffer was continuously applied into the reservoir of the PD-10 column, and the eluate was collected in plastic tubes with each tube collecting ~1 mL of the eluate, until a total of 10 mL of eluate was collected.

5 The purity of each fraction collected was assessed by iTLC-SG using 10 mM EDTA aqueous solution (pH = 5-6) as the mobile phase. Pure fraction(s) were combined to afford final product in 10mM NaOAc buffer. The product solution was analyzed by HPLC for chemical and radiochemical purity. The antibody concentration in the product solution was determined by UV absorption using a standard curve. The activity of the product solution was in turn quantified
10 using a Capintec CRC-55TW dose calibrator.

Quality control of In-111 chelation: A chelation challenge was employed using DTPA as a quality control for a purified product: A 10 mM aqueous solution of Na₅DTPA was added to a sample solution containing ¹¹¹In labeled mAb until the [DTPA]/[mAb] = 1000 - 10,000. The mixture was placed on a shake block at room temperature and 290 rpm for 30 min. The mixture
15 was spotted on iTLC-SG and developed with 10 mM EDTA aqueous solution (pH = 5-6) as the mobile phase. Free ¹¹¹In or loosely bound ¹¹¹In migrates to the solvent front and the tightly bound ¹¹¹In-mAb stays at the baseline.

Synthesis of anti-PSMA mAb-dibenzo-[1,2,3]-triazoloazocine-DFO-Zr-89

 800 µg of random azide-modified anti-PSMA mAb (average CAR between 1 and 4; the
20 same procedure can be carried out with a site-specific azido-mAb) in 10 mM HEPES 50 mM NaCl pH 7.5 or other compatible buffer was added to the solution of ⁸⁹Zr-DFO-DBCO produced as described and incubated at 37°C for 1.5 hrs before passing through a PD-10 column. The PD-10 column was pre-conditioned by passing 15 mL of isotonic saline through the column, and the washings were discarded. The reaction mixture was then pipetted into the reservoir of the pre-
25 conditioned PD-10 column, and the eluate was collected in plastic tubes. Saline was continuously applied into the reservoir of the PD-10 column, and the eluate was collected in plastic tubes with each tube collecting 0.5 mL of the eluate, until a total of 10 mL of eluate was collected. Activity of each fraction and of the material remaining on the column was determined with a dose calibrator. The product peak, usually fractions 4-7 was pooled to afford the final

product. The product solution was analyzed by HPLC for chemical and radiochemical purity. The antibody concentration in the product solution was determined by UV absorption using a standard curve. The activity of the product solution was quantified using a dose calibrator.

Synthesis of anti-PSMA mAb-DOTA-⁸⁹Zr

5 Azide-modified anti-PSMA mAb conjugate (CAR = 1 - 4), modified by random azide conjugation, in 20 mM HEPES 50 mM NaCl pH 7.5 (10.1 mg/mL, 200 μ L, $\sim$ 13.5 nmol) was added to the solution of ⁸⁹Zr-DOTA-GA-DBCO produced, as described above. The final pH of the mixture was adjusted to 7.0. The reaction solution was incubated at 37 °C for 2h, followed by purification on a PD-10 column (GE Healthcare) with either 0.9% saline, a HEPES buffer, or
10 PBS to give the product ⁸⁹Zr-DOTA-mAb in 64%.

Quality control of Zr-89 chelation: For Zr-DFO-mAbs, purified products were analyzed by HPLC only. These conjugates did not retain Zr-89 upon challenge with DTPA or EDTA. Zr-DOTA-mAbs were challenged with the addition of EDTA to 33 mM and incubated overnight at RT. Conjugates were analyzed after challenge by running over a PD-10 column and
15 found to retain 80% of the radioactivity.

Example 7: Analytical characterization of click-labeled radioconjugates

Determining radiochemical conversion

Radiochemical conversion (%RA conversion; see Tables 3-5) was determined by iTLC-SG (Instant Thin Layer Chromatography (iTLC) using a binderless, glass microfiber chromatography paper impregnated with a silica gel (SG)). %RA conversion values are calculated by dividing the integrated value of the product peak's radio signal (of different retention time of radioactive starting material and byproducts) by the value obtained when integrating all radio signal peaks present between the baseline and solvent front. This fraction of
20 product is then expressed as a percentage conversion.

For Ac-225 incorporated products, sample solutions containing $\sim$ 0.1 - 1 μ Ci of ²²⁵Ac were spotted on the baseline of an iTLC-SG strip approximately 2 cm from the bottom edge. The iTLC-SG strip was developed using a citrate-water-methanol mobile phase (20 mL 0.4 M trisodium citrate/3 mL 2 N HCl/2.3 mL MeOH), allowed to dry at room temperature, and stored

for a minimum of 6 h before analysis (reaching secular equilibrium of ^{225}Ac and all daughter nuclides). The iTLC-SG was scanned using a Bioscan AR2000 radio-TLC imaging scanner using a $^{99\text{m}}\text{Tc}$ setting.

For In-111 chelates, sample solutions containing $\sim 0.5 - 2.5 \mu\text{Ci}$ of ^{111}In were spotted on the baseline of an iTLC-SG strip, approximately 2 cm from the bottom edge. The iTLC-SG strip was developed using 10 mM sodium EDTA pH 5-6 as the mobile phase and then allowed to dry at room temperature. The dried iTLC-SG was scanned using a Bioscan AR2000 radio-TLC imaging scanner using an In-111 setting.

For Zr-89 chelates the %RA conversion was determined by dividing the activity on the product peak by the total activity including the activity in the PD-10 column, as determined by counting with a dose calibrator.

Determining radiochemical purity of radiolabeled proteins

Radiochemical purity (%RA purity; see Tables 3-4) of Ac-225 and In-111 chelates was determined by SE-HPLC (size exclusion HPLC). For Ac-225, a Tosoh TSKgel column (G3000SWxl7.8mm x 30cm, 5 μm) was used; the column was eluted with DPBS buffer (1x, without calcium and magnesium); flow rate: 0.7 mL/min; 20 min run; room temperature. After HPLC, the eluate was collected in pre-numbered vials with each vial collecting 0.5 min or 1 min of eluate fraction. The eluate-containing vials were left at room temperature for >6 h to allow ^{225}Ac reach the secular equilibrium with its daughter nuclides. The activity in each vial was then counted in a Capintec CRC-55TW well counter. The radio-chromatogram was reconstructed based on the activity in the vials.

For In-111, Tosoh TSKgel column (G3000SWxl7.8mm x 30cm, 5 μm) was used; the column was eluted with DPBS buffer (1x, without calcium and magnesium); flow rate: 0.7 mL/min; 20 min run; room temperature. Radioactivity detection was accomplished using the above HPLC system and a Perkin Elmer radio flow detector Radiomatic 625TR and equipped with a 0.5 mL flow cell using In-111 setting and Ultima FloTM M cocktail at a flow rate of 1.4 mL/min.

For Zr-89 (see Table 5), Tosoh TSKgel column (G3000SWxl7.8mm x 30cm, 5 μm) was used. The column was eluted with citrate-buffered saline; flow rate: 1 mL/min; 20 min run; room

temperature. Radioactivity detection was accomplished using the above HPLC system and a Beckman flow through detector coupled to a Bioscan Flow Count instrument.

Table 3: Actinium-225 radiolabeling of proteins

mAb	Step1*	Step 2**					CAR
		% RA conversion (iTLC)	% RA conversion (iTLC)	% RA purity (HPLC)	Spec. conc. ($\mu\text{Ci/mL}$)	Spec. ac. ($\mu\text{Ci/mg}$)	Total RA (μCi)
anti-PSMA mAb (random azide)	88%	73%	80%	17.2	74.9	17.2	3-4
anti-PSMA mAb (site specific glycan)	88%	77%	83%	7.2	102.8	7.2	2
anti-PSMA mAb (site specific MTG)	93%	70%	90%	31.3	74.2	31.3	2.0
control mAb (random azide)	92%	68%	97%	28.1	56.1	28.1	2
Panitumumab (random azide)	87%	65%	95%	24.9	66.6	24.9	3.0
Cetuximab (random azide)	91%	67%	98%	33.7	76.4	33.7	3.6
hEGF (random azide)	92%	48%	100%	19.63	1091	19.63	1.1
Transferrin (random azide)	94%	57%	78%	24.1	83.7	24.1	3.6

Trastuzumab (random azide)	94%	60%	97%	9.88	62.9	9.88	2.5
Pertuzumab (random azide)	94%	63%	92%	16.75	49.9	16.75	2.3
Anti-human HER2 (random azide)	85%	78%	94%	21.5	55.0	21.5	3.1

* Step 1 is the chelation of Actinium-225 to DOTA-GA-DBCO

** Step 2 is the click reaction the bifunctional chelate to the protein

Table 4: Indium-111 radiolabeling of proteins

mAb	Step1*	Step 2**					CAR
		% RA conversion (iTLC)	% RA conversion (iTLC)	% RA purity (HPLC)	Spec. conc. (μ Ci/mL)	Spec. ac. (μ Ci/mg)	Total RA (μ Ci)
anti-PSMA mAb (random azide)	81%	79%	98%	57.6	139.5	72	2.4
Transferrin (random azide)	79%	88%	90%	71.4	410.3	71.4	3.6
Panitumumab (random azide)	46%	79%	99%	82.0	279	82.0	3.0
Cetuximab (random azide)	74%	75%	100%	51.3	290	51.3	3.6

5 * Step 1 is the chelation of Indium-111 to DOTA-GA-DBCO

** Step 2 is the click reaction the bifunctional chelate to the protein

Table 5: Zr-89 radiolabeling of anti-PSMA mAb with DFO-DBCO

mAb	% RA conversion	% RA purity (HPLC)	Spec. conc. ($\mu\text{Ci/mL}$)	Spec. ac. ($\mu\text{Ci/mg}$)	Total RA (μCi)	CAR
anti-PSMA mAb (random azide)	47%	100%	131	1171	262	2.4

Example 8: Click reaction of modified mAb with DOTA-GA-DBCO

Random and site-specific azido-mAbs (anti-PSMA mAb, Cetuximab, Panitumumab, Trastuzumab, Pertuzumab) at 1-10 mg/mL were mixed with a 5x to 20x excess of unchelated DOTA-GA-DBCO and incubated at RT or 37°C for 1-24 hours. mAbs were desalted with Zeba desalt spin columns (Thermo) and concentrated with an Amicon centrifuge concentrator (Millipore), rediluted with buffer and concentrated again to remove any remaining DBCO-DOTA. Complete click reaction of all free azides with DBCO-DOTA was confirmed by LC-MS.

Example 9: Click reaction of modified mAb with DFO-DBCO

Random and site-specific anti-PSMA mAb azido-mAbs at 1-10 mg/mL were mixed with a 5x to 20x excess of unchelated DOTA-GA-DFO and incubated at RT or 37°C for 1-24 hours. mAbs were desalted with Zeba desalt spin columns (Thermo) and concentrated with an Amicon centrifuge concentrator (Millipore), rediluted with buffer and concentrated again to remove any remaining DBCO-DFO. Complete click reaction of all free azides with DBCO-DFO was confirmed by LC-MS.

Example 10: Cell binding (FACS)

Cell binding of azide-modified antibodies, DOTA-DBCO-azide modified antibodies, and DFO-DBCO-azide modified antibodies was compared to the parental mAbs for the conjugates described in Table 1. Cell lines expressing the mAb target were treated with antibody or conjugate in a range of concentrations, and binding was measured by flow cytometry. Panitumumab and Cetuximab conjugates were evaluated for binding to EGFR+ A431 cells.

Herceptin and Pertuzumab were evaluated for binding to HER2+ SK-BR-3 cells. Anti-PSMA mAb was evaluated for binding to PSMA+ C4-2b cells.

Cell lines: C4-2B cells, a human prostate carcinoma cell line, were obtained from Janssen Oncology (Spring House, PA). A431 cells, a human epidermoid carcinoma cell line, and SK-BR-3 cells, a human breast cancer cell line, were obtained from Janssen BioTherapeutics (Spring House, PA) with cells originally from ATCC (Manassas, VA). EGFR Receptor negative MOLM-13 Human Acute Myeloid Leukemia suspension cells were maintained in RPMI1640 + 25mM Hepes (Gibco) supplemented with 20% Heat Inactivated Fetal Bovine Serum (Gibco). Cells were grown in RPMI1640 + 25 mM Hepes (Gibco, Waltham, MA) with 10% FBS (Gibco, Waltham, MA).

Flow Cytometry: Cells were detached from flask using enzyme free cell dissociation buffer (Gibco, Waltham, MA) and strained through a 40 um filter (Falcon). 5×10^4 cells were seeded per well in a 96-well u-bottom plate. Cells were incubated with conjugated antibody or parental antibody diluted in BSA stain buffer (BD Bioscience, San Jose, CA) for 1hr at 4°C. Cells were washed twice with stain buffer. Cells were then incubated in the dark for 30 min at 4°C with AlexaFluor647 tagged anti-human IgG secondary antibody (Jackson ImmunoResearch Laboratories). Secondary antibodies were diluted 1:200 in stain buffer containing 3% donkey serum (Rockland Immunochemicals). For the last 10 minutes of the incubation SYTOX™ Green Nucleic Acid Stain (ThermoFisher) was added to cells at a final concentration of 30 nM. Cells were washed twice with stain buffer then resuspended in a final volume of 25 µL/well in stain buffer and read on the iQue Screener flow cytometer (Intellicyt). ForeCyt software was used to analyze data. Live cells were determined by excluding events with high nucleic acid stain. Mean fluorescence intensity (MFI) was determined on live cells and graphed in GraphPad Prism 7 (GraphPad Software) as log of antibody concentration vs. MFI. A non-linear regression curve fit was added to data and EC₅₀ values were calculated.

For all mAbs and conjugates tested, the parental mAbs and modified mAbs showed similar cell binding (Table 6).

Table 6: EC50s (nM) of mAbs/conjugates binding to target cells

	mAb	mAb-azide	mAb-DOTA	mAb-DFO	
Vectibix	1.3	1.3	0.9		
Cetuximab	0.6	0.2	0.4		
Herceptin	2.0	0.9	3.1		
Pertuzumab	2.5	3.3	4.4		
anti-PSMA mAb (random azide)	5.5	4.7	5.4	4.6	
anti-PSMA mAb (glycan)	0.7	0.5	0.7		
MTG samples					
	mAb	Deglycosylated	mAb-azide	mAb-DOTA	mAb-DFO
anti-PSMA mAb (MTG)	4.1	3.4	5.0	6.7	4.5

Example 11: In-111 cell binding assay

Cell binding was measured with a radiometric assay using the In-111 radiolabeled proteins described in Table 4. Anti-PSMA mAb and transferrin were tested on C4-2B cells (PSMA+ and transferrin+). Cetuximab and panitumumab were tested on A431 cells (EGFR+) and MOLM-13 cells (EGFR-).

Adherent cells were detached with Enzyme-Free Cell Dissociation Buffer (Gibco). Detached adherent cells and collected suspension cells were counted and washed with cold Stain Buffer (BD Biosciences). Varying numbers of cells in 200 μ L Stain Buffer were added to microcentrifuge tubes and placed on ice. 0.5 μ Ci of In-111 labeled protein was added to each tube and incubated for 1 hour on ice. Cells were washed with cold PBS (Gibco) to remove unbound antibody and resuspended in 500 μ L cold PBS. Samples were transferred to counting vials, and cell-associated radioactivity was determined by gamma counting (Hidex Automatic Gamma Counter).

Counts per minute (CPM) from study samples were converted to μ Ci of In-111 using CPM value using a linear regression created using known amounts of In-111 labeled protein. μ Ci Bound Values were converted to Moles Bound using the following calculation: (μ Ci Bound/Specific Activity)/MW mAb or Protein. Each data point is the mean of duplicate samples.

Click labeled In-111 anti-PSMA mAb and In-111 transferrin bound to C4-2B cells with amounts of cell-associated radioactivity increasing with increasing cell number (Fig. 3A). Click labeled In-111 anti-EGFR antibodies Panitumumab and Cetuximab bound to A431 cells with amounts of cell-associated radioactivity increasing with increasing cell number; no specific
5 binding of the click labeled anti-EGFR antibodies was detected with the negative control MOLM-13 cells (Fig. 3B).

Example 12: Indium cell uptake assay

The kinetics of internalization of In-111 click-labeled anti-PSMA mAb in C4-2B cells
10 was determined.

Cells were seeded at 3×10^6 cells per 60 mm dish (Corning) and placed in 37°C humidified CO₂ incubator overnight. Seeding media was removed and replaced with 2 mL cold Stain Buffer (BD Biosciences). Dishes were then placed on ice. 0.5 µCi In-111 labeled antibody was added to each dish and incubated for 1 hour on ice. Cells were washed with cold PBS
15 (Gibco) to remove unbound antibody from the cell surface. At various timepoints cells were assayed for surface membrane bound radioactivity and intracellular radioactivity as described below.

Surface-bound radioactivity was stripped using an acid wash stripping procedure: 1.5 mL Stripping Buffer (50 mM glycine, 150 mM NaCl pH 2.7 with pepsin (Amresco) added to 25
20 µg/mL) was added to cells, and dishes were incubated on ice for 15 minutes. Stripping Buffer was transferred to counting vials. Cells were washed with cold PBS, and washes were transferred to counting vials. Radioactivity was assayed by gamma counting (Hidex Automatic Gamma Counter). Surface membrane bound radioactivity was determined as the sum of the Stripping Buffer + PBS Washes.

Intracellular radioactivity was assayed by preparing cell lysates: After surface-bound radioactivity was stripped and cells were washed, 1.5 mL 1 M NaOH (Teknova) was added to cells, and dishes were incubated on ice for 5 minutes. Surface-Stripped Cell Lysates were transferred to counting vials. Dishes were washed with cold PBS, and washes were transferred to counting vials. Radioactivity was assayed by gamma counting (Hidex Automatic Gamma
25

Counter). Intracellular radioactivity was determined as the sum of the Surface-Stripped Cell Lysates + PBS Washes.

For Time=0 samples, cells were assayed for surface membrane bound radioactivity and intracellular radioactivity immediately after initial antibody binding on ice. For 10-minute, 30-minute, 1-hour and 2-hour samples, 3 mL cell culture media was added to each dish after initial antibody binding and dishes were placed in 37°C humidified CO₂ incubator. At each timepoint, dishes were removed from the incubator and placed on ice. Cell culture media was transferred to counting vials, and cells were washed with cold PBS. PBS washes were collected into counting vials. Cells were assayed for surface membrane bound radioactivity and intracellular radioactivity as described. At each timepoint, an Unstripped Sample was generated by incubating cells with PBS instead of Stripping Buffer prior to Cell Lysis. These samples were used to evaluate stripping efficiency and to compare results with Stripped samples.

CPM from study samples were converted to μCi In-111 using CPM value from a linear regression created using known amounts of In-111 labeled mAb. Percent Localization of In-111 mAb on cell surface membrane (stripped samples) and intracellular (lysed samples) was determined using the following formula: $\% \text{ Localized} = 100 * (\text{Sample } \mu\text{Ci} / \text{Average Total } \mu\text{Ci})$, where Total μCi refers to the addition of all collected samples including incubation media, PBS washes, glycine rinses and lysed cells. Each data point is the mean of duplicate samples.

Surface bound In-111 rapidly disappeared from the cell surface and was redistributed intracellularly. The stripping technique released ~80% of the cell surface associated radioactivity at time 0; by the end of the incubation only 20% was released by stripping and over 60% of the radioactivity was in the cell lysate (Fig. 4).

Example 13: Efficacy in mouse tumor xenograft model

Dose ranging study of anti-PSMA mAb-DOTA-Ac -225: Male NSG mice (N=8 per group) were implanted subcutaneously with 10^6 LNCaP cells, and tumors grew to 100-150 mm³. Mice were injected intravenously with a single dose of anti-PSMA mAb-azide-DOTA-²²⁵Ac or isotype control, control mAb-azide-DOTA-²²⁵Ac at a range of activities (10 nCi, 25 nCi, 70 nCi, 200 nCi). Injected dose per mouse was made up to a total of 10 μg protein with cold antibody.

Tumor size and body weight were measured twice-weekly. Animals were euthanized when tumor size exceeded 1,500 mm³, or body weight loss exceeded 20%.

Anti-PSMA mAb-DOTA-Ac-225 shows tumor growth inhibition after a single dose, particularly at the higher radioactive doses, and superior to isotype control at all doses (Fig. 5A).

All doses of the control mAb radioconjugate had similar survival curves to the vehicle control (Fig. 5B; Table 7); the anti-PSMA mAb conjugate showed a clear dose-response with survival increasing with increasing radioactive dose (Fig. 5C; Table 7). The study was terminated after 209 days, with 3 mice remaining, all from the anti-PSMA mAb 200 nCi group and showing no detectable tumors.

Table 7: Median survival

Dose	anti-PSMA mAb	Isotype control
10 nCi	61 d	46 d
25 nCi	82 d	48 d
70 nCi	92.5 d	50 d
200 nCi	126.5 d	39.5 d
Vehicle	46	

The embodiments of the invention are intended to be merely exemplary, and those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures of the invention. All such equivalents are considered to be within the scope of the present invention and are covered by the following claims.

All references (including patent applications, patents, and publications) cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

CLAIMS

I/we claim:

1. A method of labeling a polypeptide with a radiometal ion, the method comprising:
 - a. providing a modified polypeptide comprising the polypeptide covalently linked to a first click reaction partner;
 - b. providing a radiocomplex comprising the radiometal ion associated with a chelating moiety, wherein the chelating moiety comprises a chelant covalently linked to a second click reaction partner; and
 - c. contacting the modified polypeptide with the radiocomplex under a condition to allow the first click reaction partner to react with the second click reaction partner to thereby label the polypeptide with the radiometal ion.
2. The method of claim 1, wherein one of the first and second click reaction partners comprises an alkyne group, and the other click reaction partner comprises an azide, or wherein one of the first and second click reaction partners comprises an alkene group, and the other click reaction partner comprises a diene.
3. The method of claim 1 or 2, wherein the polypeptide is an antibody, or an antigen binding fragment thereof.
4. The method of claim 3, wherein the antibody is an anti-PSMA monoclonal antibody.
5. The method of any one of claims 1-4, wherein the radiometal ion is ^{225}Ac , ^{111}In or ^{89}Zr .
6. The method of any one of claims 1-4, further comprising reacting an electrophile on a side chain with a sulfhydryl group covalently linked to the first click reaction partner to obtain the modified polypeptide.

7. The method of any one of claims 1-5, wherein the modified polypeptide is a modified antibody or antigen binding fragment thereof obtained by a site-specific incorporation of the first click reaction partner.

8. The method of claim 7, wherein the modified antibody or antigen binding fragment thereof is obtained by a method comprising trimming an antibody or antigen binding fragment thereof with a bacterial endoglycosidase specific for the β -1,4 linkage between a core GlcNAc residue in a **Fc-glycosylation site of the antibody to obtain a trimmed antibody or** antigen binding fragment thereof, **and reacting the trimmed antibody or** antigen binding fragment thereof with an azide-labeled sugar, in the presence of a sugar transferase, such as GalT galactosyltransferase or GalNAc transferase, to thereby obtain the modified antibody or antigen binding fragment thereof.

9. The method of claim 8 wherein the azide-labeled sugar is UDP-N-azidoacetyl galactosamine (UDP-GalNaz) or UDP-6-azido 6-deoxy GalNAc.

10. The method of claim 8 wherein the sugar transferase is GalT galactosyltransferase or GalNAc transferase.

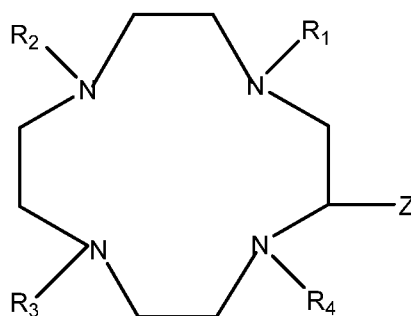
11. The method of claim 7, wherein the modified antibody or antigen binding fragment thereof is obtained by a method comprising deglycosylating an antibody or antigen binding fragment thereof with an amidase to **obtain a deglycosylated antibody or** antigen binding fragment thereof, **and reacting the deglycosylated antibody or** antigen binding fragment thereof with an azido amine in the presence of a microbial transglutaminase to thereby obtain the modified polypeptide.

12. The method of claim 11 wherein the azido amine is selected from 3-azido propylamine, 6-azido hexylamine, O-(2-Aminoethyl)-O'-(2-azidoethyl)tetraethylene glycol, O-(2-

Aminoethyl)-O'-(2-azidoethyl)pentaethylene glycol, and O-(2-Aminoethyl)-O'-(2-azidoethyl)triethylene glycol.

13. The method of claim 1, wherein the modified polypeptide comprises the polypeptide covalently linked, directly or via a linker, to an azide, tetrazine or tetrazole group.

14. The method claim 1, wherein the chelant comprises a macrocycle having the structure of formula (I):



formula (I),

wherein each of R₁, R₂, R₃ and R₄ is independently CHQCO₂X, wherein

Q is independently hydrogen, C₁-C₄ alkyl or (C₁-C₂ alkyl) phenyl, and

X is independently hydrogen, benzyl, C₁-C₄ alkyl; and

Z is (CH₂)_nY, wherein

n is 1-10, and

Y is an electrophilic or nucleophilic moiety covalently linked to the second click reaction partner;

alternatively, Z is hydrogen; and

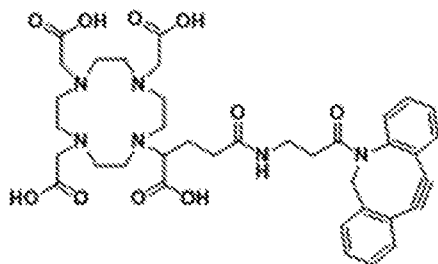
each of R₁, R₂, R₃ and R₄ is independently CHQCO₂X, wherein

Q is independently hydrogen, C₁-C₄ alkyl or (C₁-C₂ alkyl) phenyl, and

X is independently hydrogen, benzyl, C₁-C₄ alkyl, or an electrophilic or nucleophilic moiety covalently linked to the second click reaction partner;

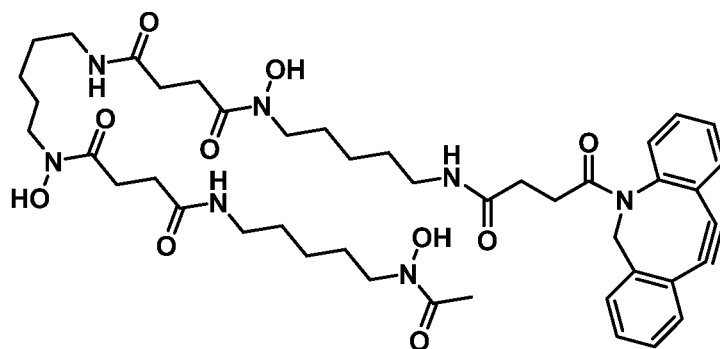
alternatively, the chelant comprises an open chain ligand.

15. The method of claim 1, wherein the chelating moiety comprises the structure of formula (II):



formula (II).

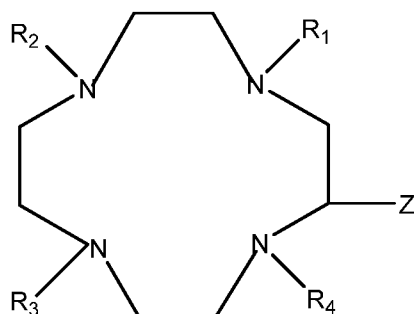
or a structure of formula (III):



Formula (III).

16. A method of labeling an antibody or an antigen binding fragment thereof with ^{225}Ac , ^{111}In or ^{89}Zr , the method comprising:
- providing a modified antibody or antigen binding fragment thereof comprising the antibody or antigen binding fragment thereof covalently linked to an azide, tetrazine or tetrazole group;
 - providing a radiocomplex comprising ^{225}Ac , ^{111}In or ^{89}Zr associated with a chelating moiety, wherein the chelating moiety comprises a chelant covalently linked to an alkyne or an alkene group; and
 - contacting the modified antibody or antigen binding fragment thereof with the radiocomplex under a condition to allow the azide, tetrazine or tetrazole group to react

with the alkyne or the alkene group to thereby label the antibody or antigen binding fragment thereof with ^{225}Ac , ^{111}In or ^{89}Zr ,
 wherein the chelant comprises a structure of formula (I):



formula (I),

wherein each of R₁, R₂, R₃ and R₄ is independently CHQCO₂X, wherein

Q is independently hydrogen, C₁-C₄ alkyl or (C₁-C₂ alkyl) phenyl, and

X is independently hydrogen, benzyl, C₁-C₄ alkyl; and

Z is (CH₂)_nY, wherein

n is 1-10, and

Y is an electrophilic or nucleophilic moiety covalently linked to the alkyne group;
 alternatively, Z is hydrogen; and

each of R₁, R₂, R₃ and R₄ is independently CHQCO₂X, wherein

Q is independently hydrogen, C₁-C₄ alkyl or (C₁-C₂ alkyl) phenyl, and

X is independently hydrogen, benzyl, C₁-C₄ alkyl, or an electrophilic or
 nucleophilic moiety covalently linked to the alkyne group,

or, alternatively, the chelant comprises an open chain ligand.

17. The method of claim 16, further comprising reacting an electrophile on a side chain with a sulfhydryl group covalently linked to the azide, tetrazine or tetrazole group to obtain the modified antibody or antigen binding fragment thereof.

18. The method of claim 16, wherein the modified antibody or antigen binding fragment thereof is obtained by a site specific incorporation of the first click reaction partner.

19. The method of claim 18, wherein the modified antibody or antigen binding fragment thereof is obtained by a method comprising trimming an antibody or antigen binding fragment thereof with a bacterial endoglycosidase specific for the β -1,4 linkage between a core GlcNac residue in a **Fc-glycosylation site of the antibody to obtain a trimmed antibody or** antigen binding fragment thereof, **and reacting the trimmed antibody or** antigen binding fragment thereof with an azide-labeled sugar in the presence of a sugar transferase, to thereby obtain the modified antibody or antigen binding fragment thereof.

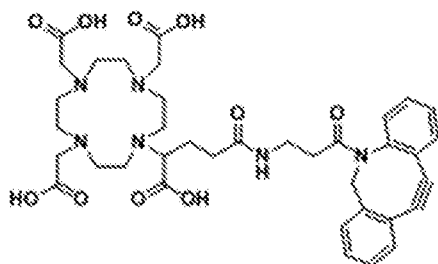
20. The method of claim 19 wherein the azide labeled sugar is UDP-N-azidoacetylgalactosamine (UDP-GalNaz) or UDP-6-azido 6-deoxy GalNAc.

21. The method of claim 19 wherein the sugar transferase is selected from GalT galactosyltransferase or GalNAc transferase.

22. The method of claim 16, wherein the modified antibody or antigen binding fragment thereof is obtained by a method comprising deglycosylating an antibody or antigen binding fragment thereof with an amidase to **obtain a deglycosylated antibody or** antigen binding fragment thereof, **and reacting the deglycosylated antibody or** antigen binding fragment thereof with an azido amine, in the presence of a microbial transglutaminase to thereby obtain the modified polypeptide.

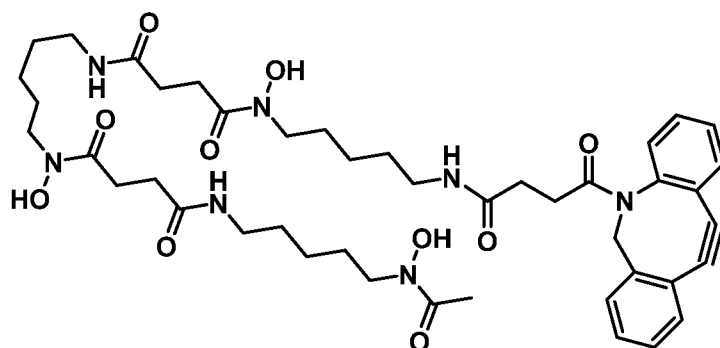
23. The method of claim 22 wherein the azido amine is selected from 3-azido propylamine, 6-azido hexylamine, O-(2-Aminoethyl)-O'-(2-azidoethyl)tetraethylene glycol, O-(2-Aminoethyl)-O'-(2-azidoethyl)pentaethylene glycol, and O-(2-Aminoethyl)-O'-(2-azidoethyl)triethylene glycol.

24. The method of 16, wherein the chelating moiety comprises the structure of formula (II):



formula (II)

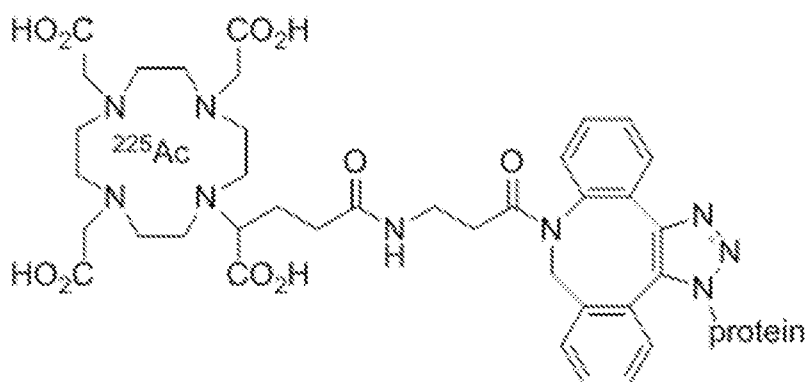
or a structure of formula (III):



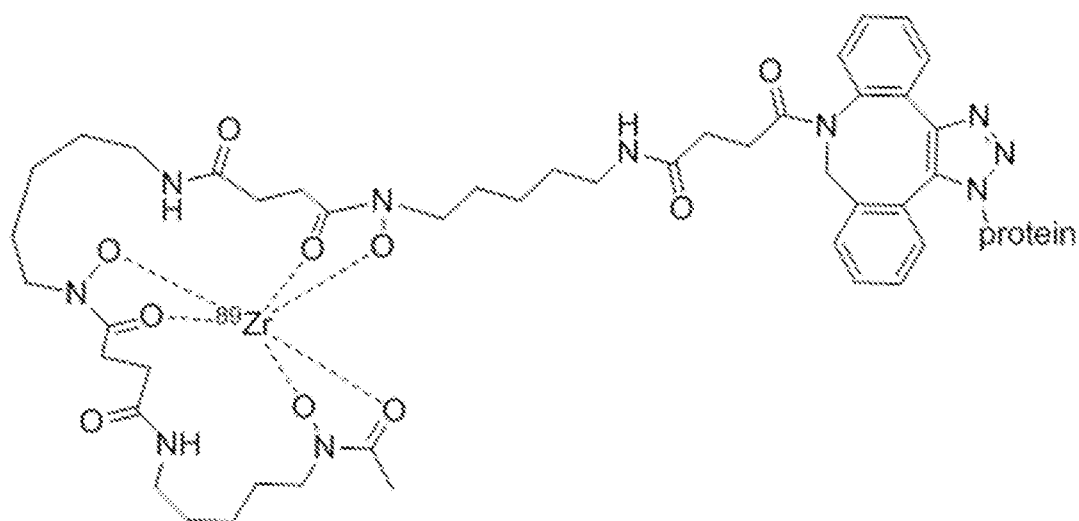
formula (III).

25. A method of dually labeling a polypeptide with two radiometal ions, the method comprising:
- providing a modified polypeptide comprising the polypeptide covalently linked to a first click reaction partner and a second click reaction partner;
 - providing a first radiocomplex comprising the first radiometal ion associated with a chelating moiety, wherein the chelating moiety comprises a chelant covalently linked to a third click reaction partner; and
 - providing a second radiocomplex comprising the second radiometal ion associated with a chelating moiety, wherein the chelating moiety comprises a chelant covalently linked to a fourth click reaction partner; and

- d. contacting the modified polypeptide with the radiocomplexes under a condition to allow the first click reaction partner to react with the third click reaction partner, and the second click reaction partner to react with the fourth click reaction partner, to thereby label the polypeptide with the first and second radiometal ions.
26. The method of claim 25, wherein one of the first and second click reaction partners comprises an alkyne group, and the other of the first and second click reaction partner comprises an azide, and wherein one of the third and fourth click reaction partners comprises an alkene group, and the other of the third and fourth click reaction partner comprises a diene, and wherein the first or second radiometal ion is a diagnostic emitter, and the other is a therapeutic emitter, or wherein both the first and second radiometal ions are therapeutic emitters.
27. A pharmaceutical composition comprising a radiolabeled polypeptide prepared by a method of claim 1 or 16 and a pharmaceutically acceptable carrier.
28. A method of treating a neoplastic disease or disorder in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 27.
29. A theranostic agent comprising a radiolabeled antibody prepared by a method of claim 1 or 16 and a pharmaceutically acceptable carrier, wherein the immunological properties of the radiolabeled antibody are preserved.
30. A theranostic agent prepared by the method of claim 1 having a structure of formula (VIII):



or formula (IX):



31. The theranostic agent of claim 29 wherein the radiometal ion is selected from ^{32}P , ^{47}Sc , ^{67}Cu , ^{77}As , ^{89}Sr , ^{90}Y , ^{99}Tc , ^{105}Rh , ^{109}Pd , ^{111}Ag , ^{131}I , ^{153}Sm , ^{159}Gd , ^{165}Dy , ^{166}Ho , ^{169}Er , ^{177}Lu , ^{186}Re , ^{188}Re , ^{194}Ir , ^{198}Au , ^{199}Au , ^{211}At , ^{212}Pb , ^{212}Bi , ^{213}Bi , ^{223}Ra , ^{225}Ac , ^{255}Fm , ^{227}Th , ^{62}Cu , ^{64}Cu , ^{67}Ga , ^{68}Ga , ^{86}Y , ^{89}Zr , or ^{111}In .

32. A combination comprising:

- a. a modified polypeptide comprising a polypeptide covalently linked to a first click reaction partner; and

- b. a radiocomplex comprising a radiometal ion associated with a chelating moiety, wherein the chelating moiety comprises a chelant covalently linked to a second click reaction partner;
wherein the combination is to be used for labeling the polypeptide with the radiometal ion.

Fig. 1

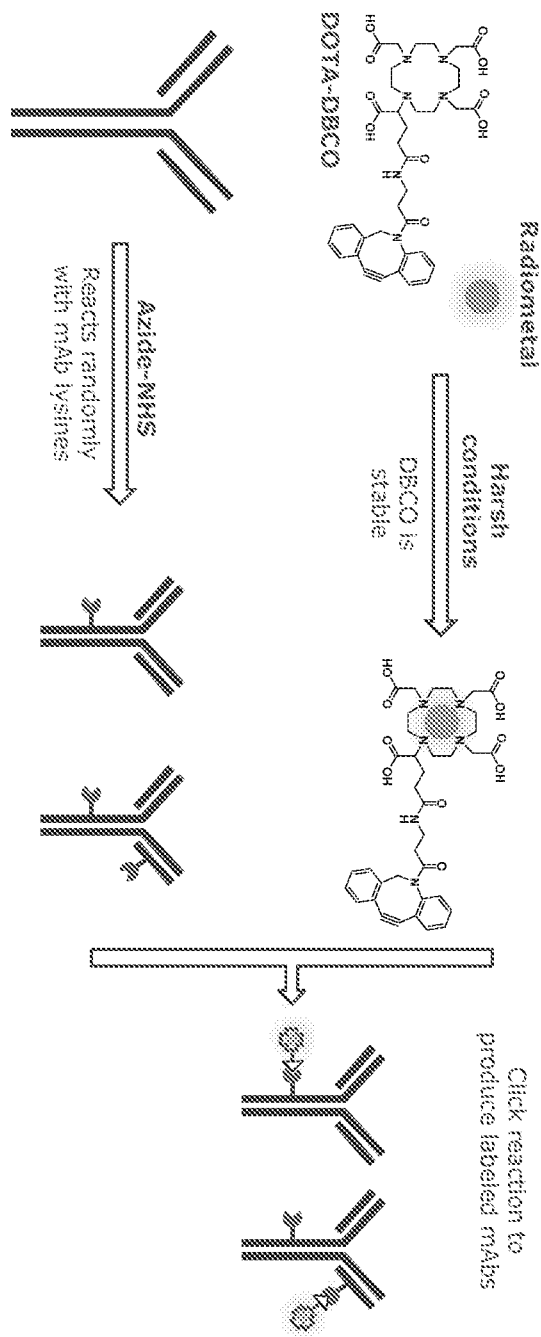
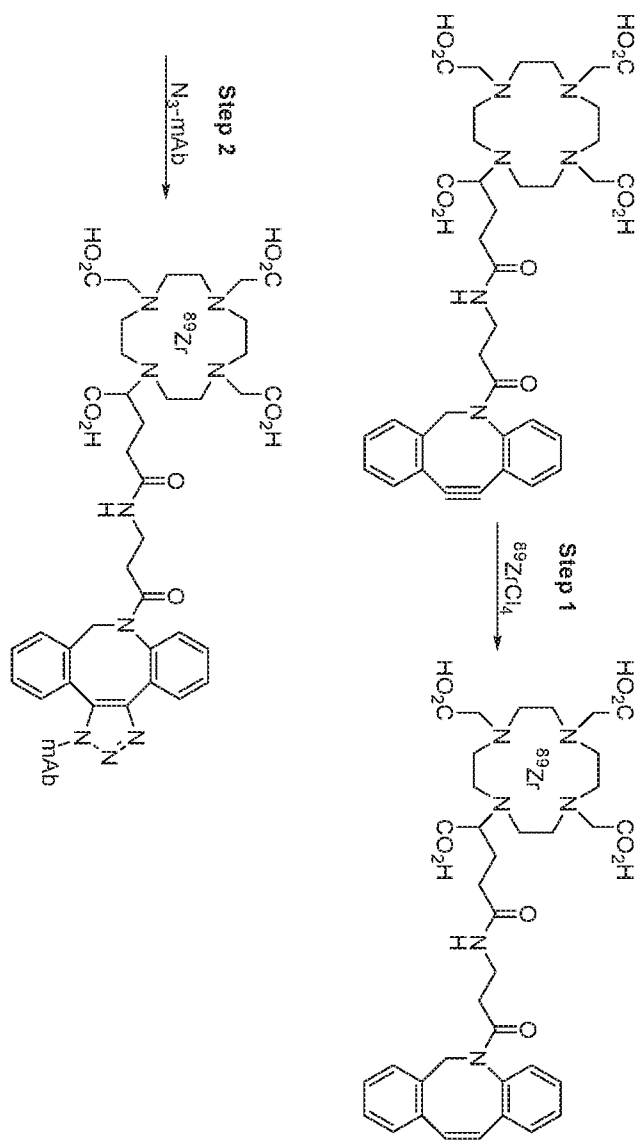


Fig. 2



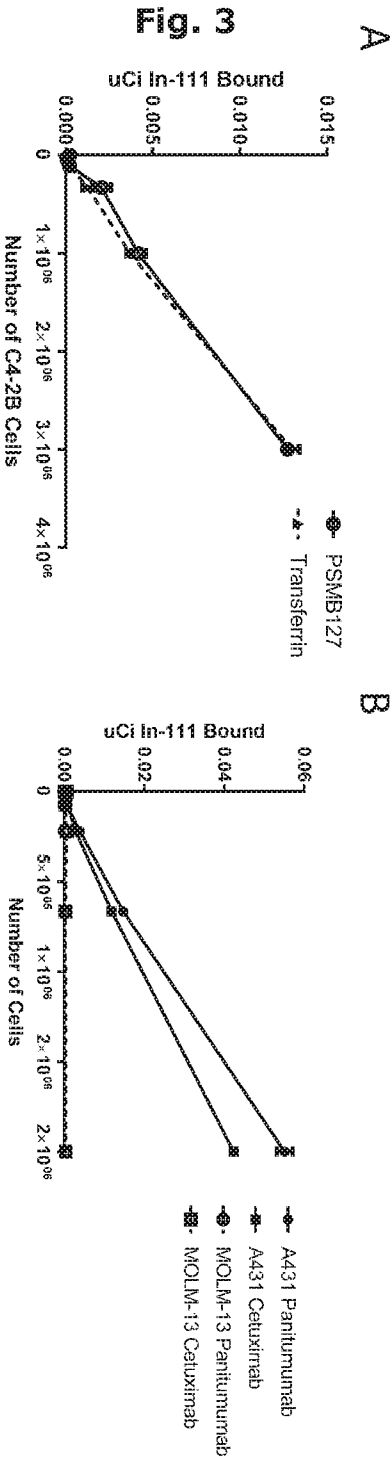


Fig. 4

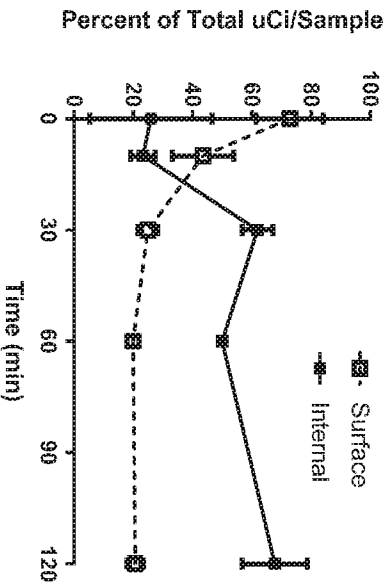


Fig. 5A

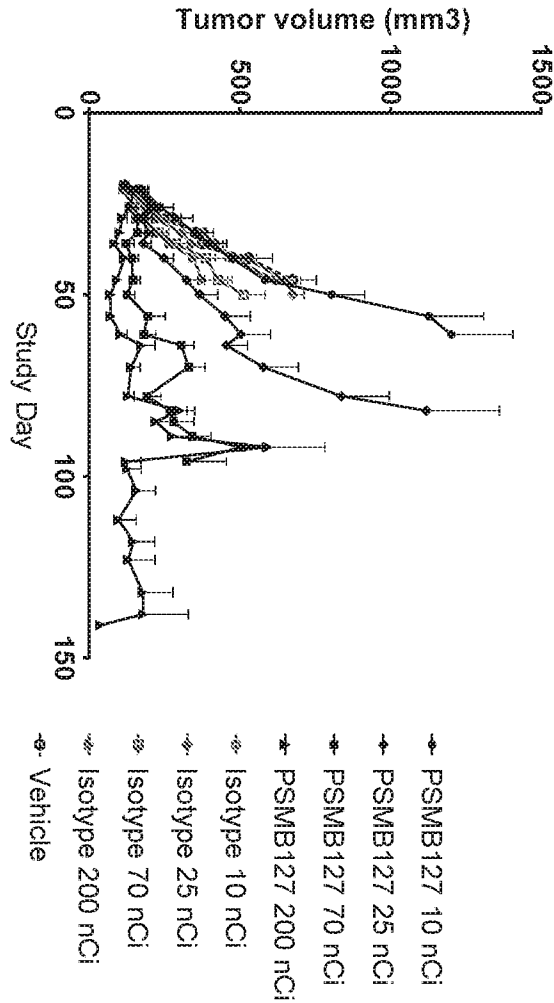


Fig. 5B

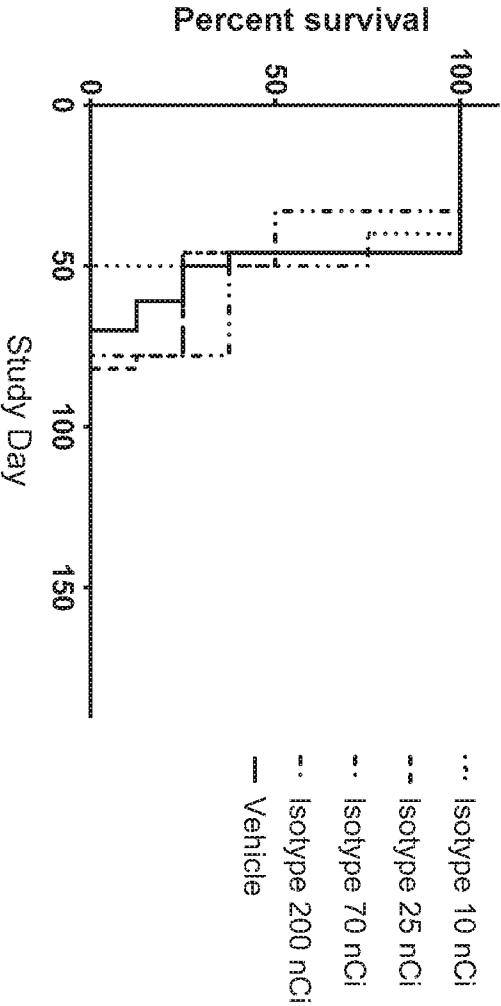
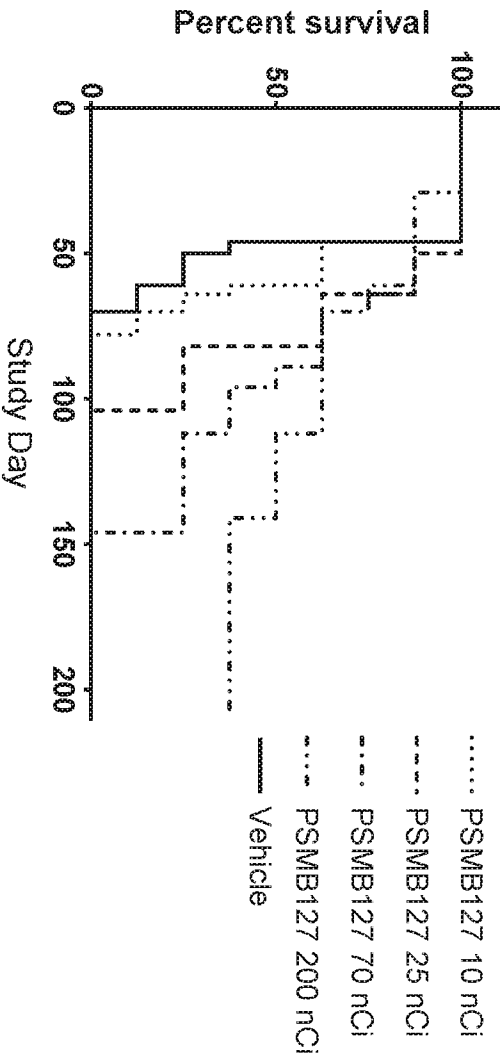


Fig. 5C



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2018/065913

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, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of 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/US2018/065913

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K51/10
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

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)

EP0-Internal, 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	LAVERMAN P ET AL: "In-depth evaluation of the cycloaddition-retro-Diels-Alder reaction for in vivo targeting with [<111>In]-DTPA-RGD conjugates", NUCLEAR MEDICINE AND BIOLOGY, ELSEVIER, NY, US, vol. 36, no. 7, 1 October 2009 (2009-10-01), pages 749-757, XP026545226, ISSN: 0969-8051, DOI: 10.1016/J.NUCMEDBIO.2009.05.001 [retrieved on 2009-07-09]	1,5
Y	figure 1 ----- -/--	2-4,6-32



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

19 March 2019

Date of mailing of the international search report

26/03/2019

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

de Nooy, Arjan

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2018/065913

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NIKKI A. THIELE ET AL: "An Eighteen-Membered Macrocyclic Ligand for Actinium-225 Targeted Alpha Therapy", ANGEWANDTE CHEMIE INTERNATIONAL EDITION, vol. 56, no. 46, 16 October 2017 (2017-10-16), pages 14712-14717, XP55543785, DE ISSN: 1433-7851, DOI: 10.1002/anie.201709532 the whole document	2-4,6-32
A	----- W. F. MAGUIRE ET AL: "Efficient 1-Step Radiolabeling of Monoclonal Antibodies to High Specific Activity with 225Ac for -Particle Radioimmunotherapy of Cancer", THE JOURNAL OF NUCLEAR MEDICINE, vol. 55, no. 9, 30 June 2014 (2014-06-30), pages 1492-1498, XP55568060, US ISSN: 0161-5505, DOI: 10.2967/jnumed.114.138347 cited in the application the whole document	1-32
A	----- WO 2015/176056 A1 (SLOAN KETTERING INST CANCER [US]; SCHEINBERG DAVID A [US]; SMITH-JONES) 19 November 2015 (2015-11-19) the whole document -----	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2018/065913

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
W0 2015176056 A1	19-11-2015	AU 2015258801 A1	12-01-2017
		CA 2949365 A1	19-11-2015
		EP 3142710 A1	22-03-2017
		US 2017112951 A1	27-04-2017
		W0 2015176056 A1	19-11-2015



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(71)申请人 詹森生物科技公司

地址 美国宾夕法尼亚州

(72)发明人 V.杜金 S.戈德堡 J.埃拉德

R.索尔特 T.M.麦克德维特

(74)专利代理机构 中国专利代理(香港)有限公司 72001

代理人 李进 黄登高

(51)Int.Cl.

A61K 51/10(2006.01)

权利要求书6页 说明书35页

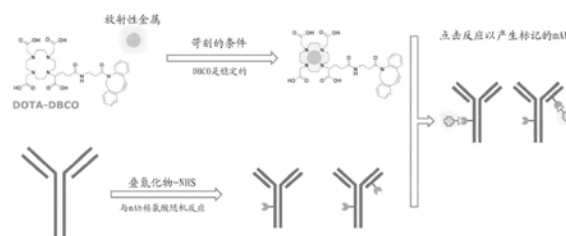
序列表10页 附图7页

(54)发明名称

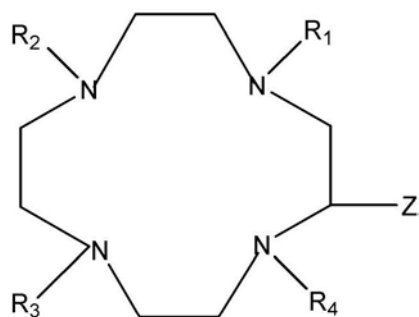
多肽的放射性标记

(57)摘要

本发明描述了使用点击化学放射性标记抗体的改善方法。还描述了与通过所述方法产生的放射性标记的抗体相关的药物组合物和用途。



1. 一种用放射性金属离子标记多肽的方法,所述方法包括:
 - a. 提供修饰的多肽,所述修饰的多肽包括共价连接到第一点击反应配偶体的多肽;
 - b. 提供放射性复合物,所述放射性复合物包含与螯合部分缔合的所述放射性金属离子,其中所述螯合部分包含共价连接到第二点击反应配偶体的螯合剂;以及
 - c. 使所述修饰的多肽与所述放射性复合物在允许所述第一点击反应配偶体与所述第二点击反应配偶体反应的条件下接触,从而用所述放射性金属离子标记所述多肽。
2. 根据权利要求1所述的方法,其中所述第一点击反应配偶体和所述第二点击反应配偶体中的一个包含炔烃基团,并且另一个点击反应配偶体包含叠氮化物,或者其中所述第一点击反应配偶体和所述第二点击反应配偶体中的一个包含烯炔基团,并且另一个点击反应配偶体包含二烯。
3. 根据权利要求1或2所述的方法,其中所述多肽为抗体或其抗原结合片段。
4. 根据权利要求3所述的方法,其中所述抗体为抗PSMA单克隆抗体。
5. 根据权利要求1至4中任一项所述的方法,其中所述放射性金属离子为 ^{225}Ac 、 ^{111}In 或 ^{89}Zr 。
6. 根据权利要求1至4中任一项所述的方法,还包括使侧链上的亲电体与共价连接到所述第一点击反应配偶体的巯基基团反应,以获得所述修饰的多肽。
7. 根据权利要求1至5中任一项所述的方法,其中所述修饰的多肽为通过位点特异性掺入所述第一点击反应配偶体来获得的修饰的抗体或其抗原结合片段。
8. 根据权利要求7所述的方法,其中所述修饰的抗体或其抗原结合片段通过这样的方法来获得,所述方法包括:用对所述抗体的Fc糖基化位点中的核心GlcNAc残基之间的 β -1,4键联具有特异性的细菌内切糖苷酶修剪抗体或其抗原结合片段,以获得修剪的抗体或其抗原结合片段,以及在糖转移酶诸如GalT半乳糖基转移酶或GalNAc转移酶的存在下使所述修剪的抗体或其抗原结合片段与叠氮化物标记的糖反应,从而获得所述修饰的抗体或其抗原结合片段。
9. 根据权利要求8所述的方法,其中所述叠氮化物标记的糖为UDP-N-叠氮基乙酰半乳糖胺(UDP-GalNAz)或UDP-6-叠氮基6-脱氧GalNAc。
10. 根据权利要求8所述的方法,其中所述糖转移酶为GalT半乳糖基转移酶或GalNAc转移酶。
11. 根据权利要求7所述的方法,其中所述修饰的抗体或其抗原结合片段通过这样的方法来获得,所述方法包括:用酰胺酶使抗体或其抗原结合片段去糖基化,以获得去糖基化的抗体或其抗原结合片段,以及在微生物转谷氨酰胺酶的存在下使所述去糖基化的抗体或其抗原结合片段与叠氮基胺反应,从而获得所述修饰的多肽。
12. 根据权利要求11所述的方法,其中所述叠氮基胺选自3-叠氮基丙胺、6-叠氮基己胺、0-(2-氨基乙基)-0'-(2-叠氮乙基)四乙二醇、0-(2-氨基乙基)-0'-(2-叠氮乙基)五乙二醇和0-(2-氨基乙基)-0'-(2-叠氮乙基)三乙二醇。
13. 根据权利要求1所述的方法,其中所述修饰的多肽包括直接或经由连接基共价连接到叠氮、四嗪或四唑基团的多肽。
14. 根据权利要求1所述的方法,其中所述螯合剂包含具有式(I)的结构的大环:



式(I),

其中 R_1 、 R_2 、 R_3 和 R_4 中的每一者独立地为 $\text{CHQC}_2\text{O}_2\text{X}$,其中

Q 独立地为氢、 C_1 - C_4 烷基或(C_1 - C_2 烷基)苯基,并且

X 独立地为氢、苄基、 C_1 - C_4 烷基;并且

Z 为 $(\text{CH}_2)_n\text{Y}$,其中

n 为1-10,并且

Y 为共价连接到所述第二点击反应配偶体的亲电或亲核部分;

另选地, Z 为氢;并且

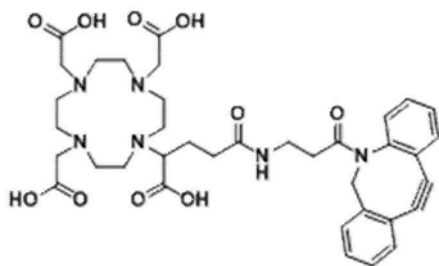
R_1 、 R_2 、 R_3 和 R_4 中的每一者独立地为 $\text{CHQC}_2\text{O}_2\text{X}$,其中

Q 独立地为氢、 C_1 - C_4 烷基或(C_1 - C_2 烷基)苯基,并且

X 独立地为氢、苄基、 C_1 - C_4 烷基或者共价连接到所述第二点击反应配偶体的亲电或亲核部分;

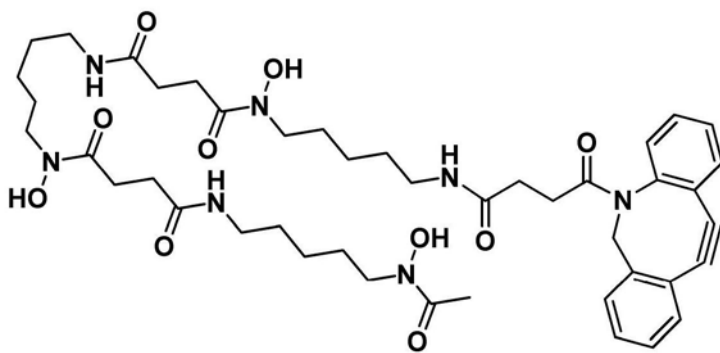
另选地,所述螯合剂包含开链配体。

15. 根据权利要求1所述的方法,其中所述螯合部分包含式(II)的结构:



式(II),

或式(III)的结构:



式(III)。

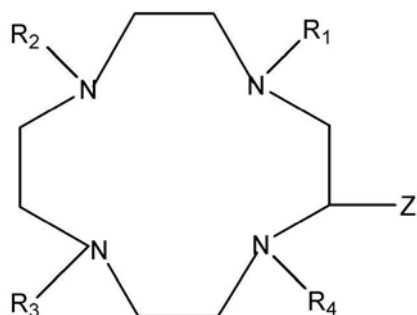
16. 一种用 ^{225}Ac 、 ^{111}In 或 ^{89}Zr 标记抗体或其抗原结合片段的方法,所述方法包括:

a. 提供修饰的抗体或其抗原结合片段,所述修饰的抗体或其抗原结合片段包含共价连接到叠氮、四嗪或四唑基团的抗体或其抗原结合片段;

b. 提供放射性复合物,所述放射性复合物包含与螯合部分缔合的 ^{225}Ac 、 ^{111}In 或 ^{89}Zr ,其中所述螯合部分包含共价连接到炔烃或烯炔基团的螯合剂;以及

c. 使所述修饰的抗体或其抗原结合片段与所述放射性复合物在允许所述叠氮、四嗪或四唑基团与所述炔烃或所述烯炔基团反应的条件下接触,从而用 ^{225}Ac 、 ^{111}In 或 ^{89}Zr 标记所述抗体或其抗原结合片段,

其中所述螯合剂包含式(I)的结构:



式(I),

其中 R_1 、 R_2 、 R_3 和 R_4 中的每一者独立地为 CHQCO_2X ,其中

Q独立地为氢、 C_1 - C_4 烷基或(C_1 - C_2 烷基)苯基,并且

X独立地为氢、苄基、 C_1 - C_4 烷基;并且

Z为 $(\text{CH}_2)_n\text{Y}$,其中

n为1-10,并且

Y为共价连接到所述炔烃基团的亲电或亲核部分;

另选地,Z为氢;并且

R_1 、 R_2 、 R_3 和 R_4 中的每一者独立地为 CHQCO_2X ,其中

Q独立地为氢、 C_1 - C_4 烷基或(C_1 - C_2 烷基)苯基,并且

X独立地为氢、苄基、 C_1 - C_4 烷基或者共价连接到所述炔烃基团的亲电或亲核部分,

或者,另选地,所述螯合剂包含开链配体。

17. 根据权利要求16所述的方法,还包括使侧链上的亲电体与共价连接到所述叠氮、四嗪或四唑基团的巯基基团反应,以获得所述修饰的抗体或其抗原结合片段。

18. 根据权利要求16所述的方法,其中所述修饰的抗体或其抗原结合片段通过位点特异性掺入所述第一点击反应配偶体来获得。

19. 根据权利要求18所述的方法,其中所述修饰的抗体或其抗原结合片段通过这样的方法来获得,所述方法包括:用对所述抗体的Fc糖基化位点中的核心GlcNAc残基之间的 β -1,4键联具有特异性的细菌内切糖苷酶修剪抗体或其抗原结合片段,以获得修剪的抗体或其抗原结合片段,以及在糖转移酶的存在下使所述修剪的抗体或其抗原结合片段与叠氮化物标记的糖反应,从而获得所述修饰的抗体或其抗原结合片段。

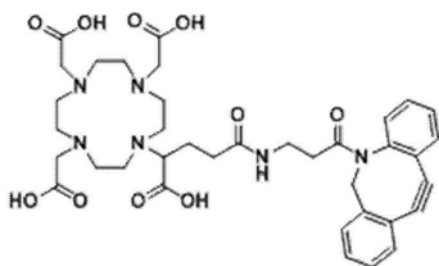
20. 根据权利要求19所述的方法,其中所述叠氮化物标记的糖为UDP-N-叠氮基乙酰半乳糖胺(UDP-GalNaz)或UDP-6-叠氮基6-脱氧GalNAc。

21. 根据权利要求19所述的方法, 其中所述糖转移酶选自GalT半乳糖基转移酶或GalNAc转移酶。

22. 根据权利要求16所述的方法, 其中所述修饰的抗体或其抗原结合片段通过这样的方法来获得, 所述方法包括: 用酰胺酶使抗体或其抗原结合片段去糖基化, 以获得去糖基化的抗体或其抗原结合片段, 以及在微生物转谷氨酰胺酶的存在下使所述去糖基化的抗体或其抗原结合片段与叠氮基胺反应, 从而获得所述修饰的多肽。

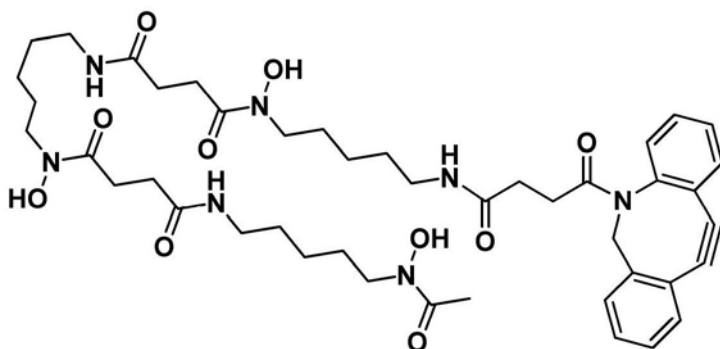
23. 根据权利要求22所述的方法, 其中所述叠氮基胺选自3-叠氮基丙胺、6-叠氮基己胺、0-(2-氨基乙基)-0'-(2-叠氮乙基) 四乙二醇、0-(2-氨基乙基)-0'-(2-叠氮乙基) 五乙二醇和0-(2-氨基乙基)-0'-(2-叠氮乙基) 三乙二醇。

24. 根据16所述的方法, 其中所述螯合部分包含式(II)的结构:



式(II)

或式(III)的结构:



式(III)。

25. 一种用两种放射性金属离子双重标记多肽的方法, 所述方法包括:

a. 提供修饰的多肽, 所述修饰的多肽包括共价连接到第一点击反应配偶体和第二点击反应配偶体的多肽;

b. 提供第一放射性复合物, 所述第一放射性复合物包含与螯合部分缔合的第一放射性金属离子, 其中所述螯合部分包含共价连接到第三点击反应配偶体的螯合剂; 以及

c. 提供第二放射性复合物, 所述第二放射性复合物包含与螯合部分缔合的第二放射性金属离子, 其中所述螯合部分包含共价连接到第四点击反应配偶体的螯合剂; 以及

d. 使所述修饰的多肽与所述放射性复合物在允许所述第一点击反应配偶体与所述第三点击反应配偶体反应并且允许所述第二点击反应配偶体与所述第四点击反应配偶体反应的条件接触, 从而用所述第一放射性金属离子和所述第二放射性金属离子标记所述多肽。

26. 根据权利要求25所述的方法, 其中所述第一点击反应配偶体和所述第二点击反应

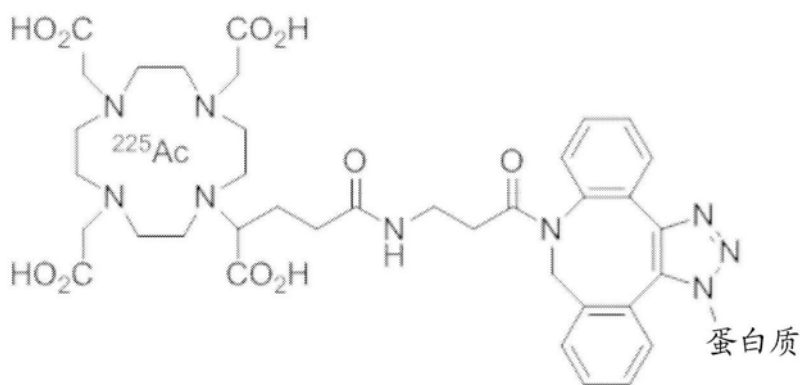
配偶体中的一个包含炔烃基团,并且所述第一点击反应配偶体和所述第二点击反应配偶体中的另一个包含叠氮化物,并且其中所述第三点击反应配偶体和所述第四点击反应配偶体中的一个包含烯烃基团,并且所述第三点击反应配偶体和所述第四点击反应配偶体中的另一个包含二烯,并且其中所述第一放射性金属离子或所述第二放射性金属离子为诊断发射体,并且另一个为治疗发射体,或者其中所述第一放射性金属离子和所述第二放射性金属离子两者为治疗发射体。

27. 一种药物组合物,包含药学上可接受的载体以及通过根据权利要求1或16所述的方法制备的放射性标记的多肽。

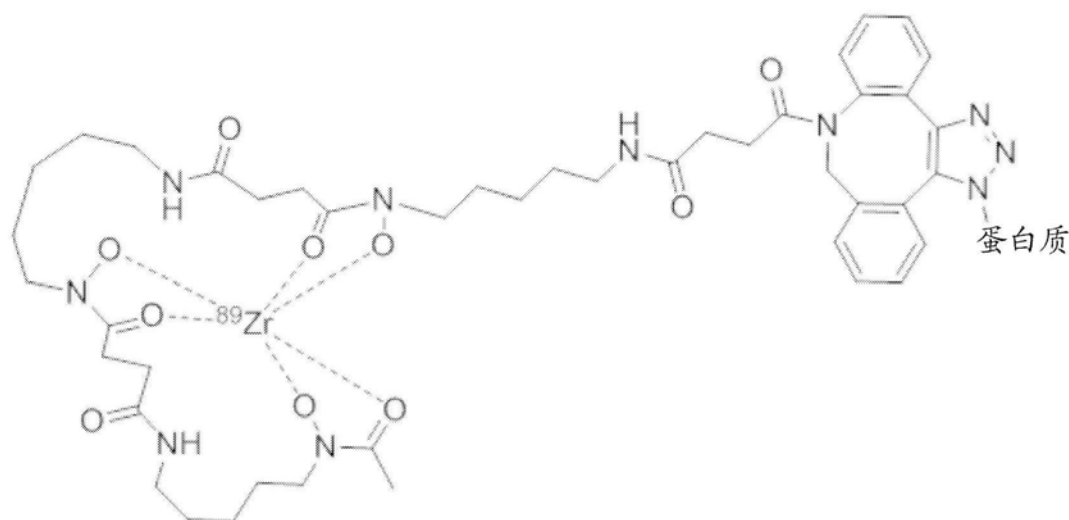
28. 一种在对其有需要的受治疗者中治疗肿瘤性疾病或障碍的方法,包括向所述受治疗者施用根据权利要求27所述的药物组合物。

29. 一种诊疗剂,包含药学上可接受的载体以及通过根据权利要求1或16所述的方法制备的放射性标记的抗体,其中保留了所述放射性标记的抗体的免疫学特性。

30. 一种通过根据权利要求1所述的方法制备的诊疗剂,所述诊疗剂具有式(VIII)的结构:



或式(IX)的结构:



31. 根据权利要求29所述的诊疗剂,其中所述放射性金属离子选自 ^{32}P 、 ^{47}Sc 、 ^{67}Cu 、 ^{77}As 、 ^{89}Sr 、 ^{90}Y 、 ^{99}Tc 、 ^{105}Rh 、 ^{109}Pd 、 ^{111}Ag 、 ^{131}I 、 ^{153}Sm 、 ^{159}Gd 、 ^{165}Dy 、 ^{166}Ho 、 ^{169}Er 、 ^{177}Lu 、 ^{186}Re 、 ^{188}Re 、 ^{194}Ir 、 ^{198}Au 、 ^{199}Au 、 ^{211}At 、 ^{212}Pb 、 ^{212}Bi 、 ^{213}Bi 、 ^{223}Ra 、 ^{225}Ac 、 ^{255}Fm 、 ^{227}Th 、 ^{62}Cu 、 ^{64}Cu 、 ^{67}Ga 、 ^{68}Ga 、 ^{86}Y 、 ^{89}Zr 或 ^{111}In 。

32. 一种组合, 包括:

- a. 修饰的多肽, 所述修饰的多肽包括共价连接到第一点击反应配偶体的多肽; 以及
 - b. 放射性复合物, 所述放射性复合物包含与螯合部分缔合的放射性金属离子, 其中所述螯合部分包含共价连接到第二点击反应配偶体的螯合剂;
- 其中所述组合将用于用所述放射性金属离子标记所述多肽。

多肽的放射性标记

[0001] 以电子方式提交的参考序列表

[0002] 本申请含有序列表,该序列表以电子方式经由EFS-Web作为ASCII格式化序列表提交,文件名为“Sequence_Listing”,创建日期为2018年12月7日,并且大小为约13.2kB。经由EFS-Web提交的该序列表是本说明书的一部分并且全文以引用方式并入本文。

[0003] 相关申请的交叉引用

[0004] 本申请根据35U.S.C. §119(e) 享有2017年12月18日提交的美国临时专利申请62/599,830的优先权,其公开内容全文以引用方式并入本文。

技术领域

[0005] 本发明涉及用于放射性标记多肽诸如抗体的方法。具体地讲,本发明涉及使用点击化学以用放射性金属离子标记多肽的方法。本发明还涉及放射性标记的多肽的药物组合物和用途。

背景技术

[0006] 发射 α 粒子的放射性核素由于其高能量与短程作用的组合,对于癌症治疗具有良好前景,提供了主要定位于肿瘤细胞的强效杀灭的可能性(Kim,Y.S.和M.W.Brechbiel,An overview of targeted alpha therapy.Tumour Biol,2012.33(3):第573-90页)。使用抗体、支架蛋白质、小分子配体、核酸适体或对癌症抗原具有特异性的其他结合部分靶向递送 α 发射体,提供了一种将放射性核素选择性递送至肿瘤以增强其效力并减轻脱靶效应的方法。在惯例上,结合部分附接到螯合剂,该螯合剂结合到发射 α 的放射性金属,以产生放射性复合物。许多此类示例使用单克隆抗体(mAb)作为靶向配体,以产生称为放射性免疫缀合物的化合物。

[0007] 镭-225 (^{225}Ac) 是用于医学应用的特别受关注的发射 α 的放射性同位素(Miederer等人,Realizing the potential of the Actinium-225 radionuclide generator in targeted alpha particle therapy applications.Adv Drug Deliv Rev,2008.60(12):71-82)。 ^{225}Ac 的10天半衰期足够长以有利于放射性缀合物产生,但也足够短以匹配递送溶媒诸如抗体的循环药代动力学。因此, ^{225}Ac 放射性免疫缀合物特别受关注。此外, ^{225}Ac 在达到稳定同位素 ^{209}Bi 之前最终发射4个 α 粒子的一系列步骤中衰减,从而增加效力。另一种用于医学应用的受关注的放射性同位素为镥177 (^{177}Lu),其发射适用于成像的 γ 辐射和适用于放射治疗的中能 β 辐射。已显示 ^{177}Lu 标记的肽表现出减少的正常组织损伤,并且 ^{177}Lu 标记使得可以使用单一放射性标记剂进行治疗和成像(Kwekkeboom DJ等人,[^{177}Lu -DOTA0Tyr3]octreotate:comparison with[^{111}In -DTPA0]octreotide in patients.Eur J Nucl Med.2001;28:第1319-1325页)。用于治疗应用的其他放射性同位素包括例如 β 或 α 发射体,诸如例如钍、镭、 ^{32}P 、 ^{47}Sc 、 ^{67}Cu 、 ^{77}As 、 ^{89}Sr 、 ^{90}Y 、 ^{99}Tc 、 ^{105}Rh 、 ^{109}Pd 、 ^{111}Ag 、 ^{131}I 、 ^{153}Sm 、 ^{159}Gd 、 ^{165}Dy 、 ^{166}Ho 、 ^{169}Er 、 ^{186}Re 、 ^{188}Re 、 ^{194}Ir 、 ^{198}Au 、 ^{199}Au 、 ^{211}At 、 ^{212}Pb 、 ^{212}Bi 、 ^{213}Bi 、 ^{223}Ra 、 ^{255}Fm 和 ^{227}Th 。用于成像应用的其他放射性同位素包括发射 γ 的放射性同位素,诸如例如 ^{62}Cu 、 ^{64}Cu 、 ^{67}Ga 、

^{68}Ga 、 ^{86}Y 、 ^{89}Zr 和 ^{111}In 。

[0008] 先前的临床和临床前程序主要使用1,4,7,10-四氮杂环十二烷-1,4,7,10-四乙酸(DOTA)来进行铜螯合。然而,已知的是,铜的DOTA螯合可以是具有挑战性的(Deal, K.A. 等人, Improved in vivo stability of actinium-225 macrocyclic complexes. *J Med Chem*, 1999. 42 (15): 第2988-92页), 并且通常需要苛刻的条件或每个抗体高水平的DOTA。因此,已利用了两种不同的方法,称为“一步”和“两步”放射性标记方法,每种方法具有其自身的缺点。

[0009] 首先开发“两步”法,其包括涉及铜的2个化学步骤(McDevitt, M.R. 等人, Tumor therapy with targeted atomic nanogenerators. *Science*, 2001. 294 (5546): 第1537-40页)。通过双官能螯合剂(BFC) DOTA-异硫氰酸酯(DOTA-SCN)在pH 4.5-5下,使用2M乙酸盐缓冲液在55℃至60℃下以高放射性化学收率(约95%)螯合 ^{225}Ac 30min。随后,使 ^{225}Ac -DOTA-SCN与靶向抗体反应,以产生放射性免疫缀合物。两步法的主要缺点是约90%的SCN不能在标记条件下存活,因此约90%的输入 ^{225}Ac 缀合到DOTA的非反应形式,该DOTA的非反应形式不能缀合到抗体。这不仅导致收率低(通常仅约10%)且成本更高,而且导致可限制最终缀合物的功效的比活性降低。

[0010] 最近开发了“一步”法用于铜(Maguire, W.F. 等人, Efficient 1-step radiolabeling of monoclonal antibodies to high specific activity with ^{225}Ac for alpha-particle radioimmunotherapy of cancer. *J Nucl Med*, 2014. 55 (9): 第1492-8页)。该方法仅具有1个涉及铜的化学反应步骤。首先将DOTA-SCN缀合到抗体。然后,在温和条件(37℃、pH 7.5)下将 ^{225}Ac 螯合到DOTA-mAb,从而实现高达80%的放射性化学收率。然而,有必要缀合高水平的DOTA(每个抗体约10或更多)以实现高收率。高螯合剂:抗体比率(CAR),在这种情况下为高DOTA:Ab比率(DAR),物质更可能具有受损的免疫反应性;此外,虽然平均DAR可为10,但是 ^{225}Ac 可能在甚至高于平均值的比率下与群体螯合。因此,该方法存在将 ^{225}Ac 连接到抗体螯合剂缀合物的最小活性部分的风险。此外,有必要在无金属条件下处理抗体和DOTA-mAb缀合物,以避免常见金属诸如铁、锌和铜的螯合,这在生产过程中引入了重大挑战。

[0011] 点击化学是由Sharpless于2001年引入的化学方法,并且其描述了通过将小单元连接在一起而被调制为快速且可靠地生成物质的化学。参见例如Kolb, Finn and Sharpless *Angewandte Chemie International Edition* (2001) 40:2004-2021; Evans, *Australian Journal of Chemistry* (2007) 60:384-395)。偶合反应(其中的一些可被分类为“点击化学”)包括但不限于由活化的酸或卤化酰基形成酯、硫酯、酰胺(例如,诸如肽偶合);亲核置换反应(例如,诸如卤化物的亲核置换或应变环系的开环);叠氮-炔Huisgen环加成(例如,叠氮化物与炔烃之间的1,3-偶极环加成以形成1,2,3-三唑连接基);巯基炔加成;亚胺形成;四嗪与反式环辛烯(TCO)之间的狄尔斯-阿尔德反应(Diels-Alder reaction);以及迈克尔加成(Michael addition)(例如,马来酰亚胺加成)。

[0012] 炔烃与叠氮化物之间的点击化学反应通常需要添加铜催化剂以促进1,3-环加成反应,并且称为铜催化的叠氮-炔环加成(CuAAC)反应。然而,环辛炔或环辛炔衍生物与叠氮化物之间的点击化学反应通常不需要添加铜催化剂,而是经由应变促进的叠氮-炔环加成(SPAAC)进行(Debets, M.F. 等人, Bioconjugation with strained alkenes and

alkynes. Acc Chem Res, 2011. 44 (9) : 第805-15页)。

[0013] 位点特异性已成为抗体药物缀合物 (ADC) 领域中的关键聚焦区域 (Agarwal, P. 和 C.R. Bertozzi, Site-specific antibody-drug conjugates: the nexus of bioorthogonal chemistry, protein engineering, and drug development. Bioconjug Chem, 2015. 26 (2) : 第176-92页), 因为已证明, 与随机缀合相比, 用位点特异性方法可增加 ADC 的功效率和安全性。据认为, 放射性免疫缀合物可实现类似的安全性和功效有益效果。

[0014] 如上所指出, 本领域仍需要产生具有高比活性和高收率的稳定放射性免疫缀合物的有效方法。

发明内容

[0015] 本发明通过提供用于使用点击化学放射性标记多肽诸如抗体的方法来满足此需要。在本发明的方法中, 将叠氮化物修饰的抗体以及包含与含有炔烃基团的螯合部分缔合的放射性金属离子的放射性复合物用于点击化学反应中, 以产生具有低螯合剂: 抗体比率 (CAR) 和高放射性化学收率的稳定放射性免疫缀合物, 同时需要减少放射性金属的使用并且在用于产生初始放射性复合物的步骤中仅需要无金属条件。本发明的方法简化了用于产生具有增加的安全性、功效和均匀度的放射性免疫缀合物的先前方法。

[0016] 在一个总体方面, 本发明涉及一种用放射性金属离子标记多肽的方法, 所述方法包括:

[0017] a. 提供修饰的多肽, 所述修饰的多肽包括共价连接到第一点击反应配偶体的多肽;

[0018] b. 提供放射性复合物, 所述放射性复合物包含与螯合部分缔合的所述放射性金属离子, 其中所述螯合部分包含共价连接到第二点击反应配偶体的螯合剂; 以及

[0019] c. 使所述修饰的多肽与所述放射性复合物在允许所述第一点击反应配偶体与所述第二点击反应配偶体反应的条件下接触, 从而用所述放射性金属离子标记所述多肽。

[0020] 在另一个总体方面, 本发明涉及一种药物组合物, 所述药物组合物包含药学上可接受的载体以及通过本发明的方法制备的放射性标记的多肽。

[0021] 在另一个总体方面, 本发明涉及一种在对其有需要的受治疗者中治疗肿瘤性疾病或障碍的方法, 所述方法包括向所述受治疗者施用本发明的药物组合物。

[0022] 在另一个总体方面, 本发明涉及一种组合或试剂盒, 所述组合或试剂盒包含:

[0023] a. 修饰的多肽, 所述修饰的多肽包括共价连接到第一点击反应配偶体的多肽; 以及

[0024] b. 放射性复合物, 所述放射性复合物包含与螯合部分缔合的放射性金属离子, 其中所述螯合部分包含共价连接到第二点击反应配偶体的螯合剂;

[0025] 其中所述组合或试剂盒将用于用所述放射性金属离子标记所述多肽。

[0026] 在其他总体方面, 本发明涉及一种治疗或诊断剂 (“诊疗剂”), 所述治疗或诊断剂包含通过本发明的方法制备的放射性标记的多肽。

附图说明

[0027] 当结合附图阅读时, 将更好地理解上述发明内容以及下文本发明的详细描述。应

当理解,本发明不限于附图中示出的精确实施方案。

[0028] 在附图中:

[0029] 图1示出根据本发明的方法的放射性标记抗体的示意图;图中示出随机缀合,并且当叠氮化物与单克隆抗体(mAb)位点特异性缀合时,使用类似的放射性标记方案;

[0030] 图2示出根据本申请的实施方案,经由点击化学对⁸⁹Zr-DOTA-mAb进行改善的两步制备的合成方案;

[0031] 图3示出In-111放射性免疫缀合物的细胞结合:结合的放射性随着细胞数量的增加而增加;具体地讲:

[0032] 图3A示出根据本申请的实施方案,通过PSMA结合抗体(“PSMB127”)In-111放射性免疫缀合物和人转铁蛋白In-111放射性缀合物来与人前列腺癌细胞系C4-2B(PSMA+,转铁蛋白受体+)结合;并且

[0033] 图3B示出根据本申请的实施方案,通过EGFR-结合抗体西妥昔单抗(cetuximab)和帕尼单抗(panitumumab)In-111放射性免疫缀合物来与人表皮样癌细胞系A431(EGFR+)结合,以及这些缀合物缺乏与对照(EGFR-)人AML细胞系MOLM-13的结合;

[0034] 图4示出根据本申请的实施方案,用抗PSMA mAb In-111放射性免疫缀合物治疗的人前列腺癌细胞系C4-2B中In-111的细胞内化的动力学;表面结合的In-111从细胞表面快速消失并重新分布在细胞内;并且

[0035] 图5示出小鼠肿瘤异种移植研究的结果;将人前列腺癌LNCaP细胞植入小鼠;当肿瘤达到100mm³时,用一系列活性的根据本申请的实施方案的单剂量的点击放射性标记的抗PSMA mAb(“PSMB127”)锕放射性缀合物或结合在该系统放射性缀合物中不存在的病毒靶的同种型对照即人IgG4抗体治疗小鼠;具体地讲:

[0036] 图5A示出每组的肿瘤体积;绘制尺寸,直到剩余低于一半的组;

[0037] 图5B示出对照mAb组的存活曲线;并且

[0038] 图5C示出抗PSMA mAb组的存活曲线。

具体实施方式

[0039] 背景以及说明书全篇中引用或描述了各种出版物、文章和专利;这些参考文献中的每一者全文均以引用方式并入本文。本说明书中包括的对文件、行为、材料、装置、文章等的讨论旨在为本发明提供上下文。此类讨论并不是承认这些事项中的任一事项或全部事项均相对于所公开或受权利要求书保护的任何发明形成现有技术的一部分。

[0040] 除非另有定义,否则本文所用的所有技术和科学术语均具有与本发明所属领域的普通技术人员通常所理解的相同的含义。否则,本文引用的某些术语具有本说明书中所述的含义。本文引用的所有专利、公布的专利申请和出版物均以引用方式并入本文,如同在本文中进行了充分阐述。

[0041] 应该注意的是,除非上下文清楚地指明,否则如本文和所附权利要求中所用的单数形式“一个/一种”和“所述/该”包括复数引用物。

[0042] 贯穿本说明书和随后的权利要求书,除非上下文另有要求,否则词语“包括”以及诸如“包含”和“含有”的变型形式将被理解为暗示包括所陈述的整数或步骤或者整数或步骤的组,但不排除任何其他整数或步骤或者整数或步骤的组。当在本文使用时,术语“包含”

可用术语“含有”或“包括”替代,或者有时在本文使用时用术语“具有”替代。

[0043] 当在本文使用时,“由.....组成”排除权利要求要素中未指定的任何要素、步骤或成分。当在本文使用时,“基本上由.....组成”不排除没有实质上影响权利要求的基本和新颖特征的材料或步骤。每当在本文中用于本发明的一个方面或实施方案的情境时,任何前述术语“包含”、“含有”、“包括”和“具有”均可用术语“由.....组成”或“基本上由.....组成”替代以改变本公开的范围。

[0044] 如本文所用,多个列举的要素之间的连接术语“和/或”被理解为包含单个选项和组合选项两者。例如,在两个要素由“和/或”连接的情况下,第一种选项是指在没有第二个要素的情况下适用第一个要素。第二种选项是指在没有第一个要素的情况下适用第二个要素。第三种选项是指适合一起使用第一要素和第二要素。这些选项中的任一种均被理解为落在含义内,并且因此满足如本文所用的术语“和/或”的要求。多于一种选项的并行适用性也被理解为落在含义内,并且因此满足术语“和/或”的要求。

[0045] 为了帮助本申请的读者,已将说明书分成各个段落或章节,或者涉及本申请的各个实施方案。这些分割不应被认为是将段落或章节或实施方案的实质与另一段落或章节或实施方案的实质拆开。相反,本领域技术人员将理解,本说明书具有广泛的应用,并且涵盖可设想到的各个章节、段落和句子的所有组合。对任何实施方案的讨论仅意图是示例性的,并且不旨在表明本公开(包括权利要求书)的范围限于这些示例。

[0046] 多肽的点击放射性标记

[0047] 与已知程序相比,本发明的方法提供了一种用于产生适用于例如在对其有需要的受治疗者(例如,人)中进行医学应用的放射性免疫缀合物的改善方法。具体地讲,本文所述的方法通过提供用于高收率螯合金属离子(包括但不限于 ^{225}Ac 、 ^{111}In 和 ^{89}Zr)以及用于低DAR的工艺解决了当前方法的主要限制。本发明允许产生单一批次的叠氮化物标记的多肽,诸如叠氮化物-mAb缀合物,然后可将该缀合物用于产生放射性标记的诊断(例如,当用 ^{89}Zr 或 ^{111}In 标记时)或治疗(例如,当用 ^{225}Ac 标记时)目的,其中放射性标记附接在该批次的叠氮化物标记的多肽内的一个或多个相同位点处,该多肽可通过位点特异性修饰或通过随机叠氮化物缀合获得。例如,在随机叠氮化物缀合的情况下,可使用本发明的点击化学对一批包含单一分布的叠氮化物修饰的位点的叠氮化物标记的多肽的样品进行放射性标记以用于不同的目的。

[0048] 依赖于点击化学并称为“点击放射性标记”的本发明的方法涉及(1)获得修饰的多肽,诸如抗体,其包含第一点击化学反应配偶体,例如叠氮化物部分;(2)获得放射性复合物,该放射性复合物包含与螯合部分缔合的放射性金属离子(例如, ^{225}Ac 、 ^{111}In 或 ^{89}Zr),其中螯合部分包含共价连接到第二点击化学反应配偶体(例如炔基团)的螯合剂,该复合物诸如DOTA-二苯并环辛炔(DOTA-DBCO)或去铁胺-DBCO(DFO-DBCO);以及(3)在修饰的肽的点击化学反应配偶体与放射性复合物之间进行反应,诸如叠氮化物部分与炔基团之间的应变促进的炔-叠氮环加成(SPAAC)。

[0049] 本发明的方法允许放射性金属在低或高pH和/或高温条件下螯合以使效率最大化,这可在没有使炔基反应配偶体失活的风险的情况下实现。叠氮基-mAb与放射性复合物之间的有效螯合和有效SPAAC反应允许以高放射性化学收率产生放射性免疫缀合物,即使叠氮化物:mAb比率低。在本发明的方法中,必须排除痕量金属的唯一步骤是放射性金属离

子与螯合部分的螯合;抗体产生、纯化和缀合步骤不需要在无金属条件下进行。

[0050] 如本文所用,术语“点击化学”是指由Sharpless引入的化学理念,其描述了通过将包含反应性基团的小单元连接在一起而被调制为快速且可靠地生成共价键的化学(参见Kolb等人,同上)。点击化学不是指特定反应,而是指包括但不限于模拟自然界中存在的反应的反应的概念。在一些实施方案中,点击化学反应是模块化的,范围广泛,给出高化学收率,生成无害的副产物,是立体特异性的,表现出大的热力学驱动力以有助于与单一反应产物反应,和/或可在生理条件下进行。在一些实施方案中,点击化学反应表现出高原子经济性,可在简单的反应条件下进行,使用易得的起始材料和试剂,不使用毒性溶剂或者使用良性或易于去除的溶剂,诸如水,和/或通过非色谱方法诸如结晶或蒸馏提供简单的产物分离。在某些实施方案中,点击化学反应是叠氮化物($-N_3$)与炔烃或炔烃部分之间的Huisgen环加成或1,3-偶极环加成,以形成1,2,4-三唑连接基。

[0051] 在一个总体方面,本发明涉及一种用放射性金属离子标记多肽、核酸适体或小分子配体的方法,所述方法包括:

[0052] a. 提供修饰的多肽,所述修饰的多肽包括共价连接到第一点击反应配偶体的多肽;

[0053] b. 提供放射性复合物,所述放射性复合物包含与螯合部分缔合的所述放射性金属离子,其中所述螯合部分包含共价连接到第二点击反应配偶体的螯合剂;以及

[0054] c. 使所述修饰的多肽与所述放射性复合物在允许所述第一点击反应配偶体与所述第二点击反应配偶体反应的条件接触,从而用所述放射性金属离子标记所述多肽。

[0055] 如本文所用,术语“多肽”是指由经由肽键连接的氨基酸残基、相关的天然存在的结构变体及其合成的非天然存在的类似物构成的聚合物。该术语是指具有任何尺寸、结构或功能的多肽。通常,多肽为至少三个氨基酸长。多肽可为天然存在的、重组的、或合成的、或它们的任何组合。合成多肽可例如使用自动多肽合成器合成。根据优选的实施方案,多肽为抗体,优选地单克隆抗体或其片段,诸如其抗原结合片段。根据优选的实施方案,抗体或其片段对癌症抗原具有特异性。根据其他实施方案,多肽为工程化结构域或支架蛋白质。

[0056] 如本文所用,术语“抗体”或“免疫球蛋白”广义地使用并且包括免疫球蛋白或抗体分子,包括多克隆抗体、单克隆抗体(包括鼠科、人、人适应的(human-adapted)、人源化和嵌合单克隆抗体)及其抗原结合片段。

[0057] 一般来讲,抗体是对特定抗原(在本文中称为“靶”)表现出结合特异性的蛋白质或肽链。抗体结构是众所周知的。根据重链恒定结构域氨基酸序列,可将免疫球蛋白指定为五种主要种类,即IgA、IgD、IgE、IgG和IgM。IgA和IgG进一步亚分类为同种型IgA1、IgA2、IgG1、IgG2、IgG3和IgG4。因此,本发明的抗体可为五种主要种类或对应的亚类中的任一种。优选地,本发明的抗体为IgG1、IgG2、IgG3或IgG4。基于其恒定结构域的氨基酸序列,可将任何脊椎物种的抗体轻链指定为两种完全不同的类型即 κ 和 λ 中的一种。因此,本发明的抗体可含有 κ 或 λ 轻链恒定结构域。根据特定实施方案,本发明的抗体包括来自小鼠抗体或人抗体的重链和/或轻链恒定区。四种IgG亚类中的每一种具有不同的生物功能,这些生物功能被称为效应子功能。这些效应子功能通常通过与Fc受体(Fc γ R)的相互作用或者通过结合C1q并固定补体来介导。结合Fc γ R可导致抗体依赖性细胞介导的细胞溶解,而结合补体因子可导致补体介导的细胞裂解。可用于本发明的抗体可不具有效应子功能或具有最小效应

子功能,但保留其结合FcRn的能力。

[0058] 如本文所用,术语“抗原结合片段”是指抗体片段,诸如例如双抗体、Fab、Fab'、F(ab')₂、Fv片段、二硫键稳定的Fv片段(dsFv)、(dsFv)₂、双特异性dsFv(dsFv-dsFv')、二硫键稳定的双抗体(ds双抗体)、单链抗体分子(scFv)、单结构域抗体(sdab)、scFv二聚体(二价双抗体)、由包含一个或多个CDR的抗体的一部分形成的多特异性抗体、骆驼化单结构域抗体、纳米抗体、结构域抗体、二价结构域抗体或结合至抗原但不包含完整抗体结构的任何其他抗体片段。抗原结合片段能够结合至与亲本抗体或亲本抗体片段结合的抗原相同的抗原。如本文所用,术语“单链抗体”是指本领域中的常规单链抗体,这种单链抗体包含通过约15至约20个氨基酸的短肽连接的重链可变区和轻链可变区。如本文所用,术语“单结构域抗体”是指本领域中的常规单结构域抗体,这种单结构域抗体包含重链可变区和重链恒定区或仅包含重链可变区。

[0059] 如本文所用,术语“支架”或“支架蛋白质”是指具有靶结合结构域并且可结合到靶的任何蛋白质。支架含有“框架”,该框架在很大程度上是结构化的,以及“结合结构域”,该结合结构域与靶接触并提供特异性结合。支架的结合结构域不需要由支架的一个连续序列限定。在某些情况下,支架可为较大结合蛋白质的一部分,该较大结合蛋白质本身可为含有多个支架的多聚体结合蛋白质的一部分。某些结合蛋白质可为双特异性或多特异性的,因为其可结合到两个或更多个不同的表位。支架可来源于单链抗体,或支架可不来源于抗体。

[0060] 根据本公开,可使用本领域技术人员已知的用于对多肽进行化学或酶修饰的任何方法来将本发明的多肽共价连接到第一点击反应配偶体。与存在于每条多肽链的N端和赖氨酸残基的侧链中的伯胺反应的胺反应性基团可在用于随机修饰多肽的方法中使用。适用于本发明中的胺反应性基团的示例包括但不限于N-羟基琥珀酰亚胺(NHS)、取代的NHS(诸如磺基-NHS)、异硫氰酸酯以及四氟苯基酯和全氟苯基酯。与存在于半胱氨酸残基的侧链中的硫醇或巯基反应的硫醇反应性基团可在用于随机修饰多肽的方法中使用。适用于本发明中的硫醇反应性基团的示例包括但不限于马来酰亚胺、卤代乙酰基和苯基恶二唑酮。根据优选的实施方案,修饰的多肽通过使侧链(优选地赖氨酸的氨基侧链)与共价连接到第一点击反应配偶体(例如NHS-叠氮化物)的亲电体反应来获得。

[0061] 本发明的方法还允许产生位点特异性放射性标记的多肽。本发明的点击放射性标记方法通过利用已建立的方法将叠氮基团位点特异性地安装在抗体上来有利于放射性免疫缀合物的位点特异性产生(Li,X.等人,Preparation of well-defined antibody-drug conjugates through glycan remodeling and strain-promoted azide-alkyne cycloadditions. *Angew Chem Int Ed Engl*, 2014. 53(28): 第7179-82页; Xiao, H. 等人, Genetic incorporation of multiple unnatural amino acids into proteins in mammalian cells. *Angew Chem Int Ed Engl*, 2013. 52(52): 第14080-3页)。以位点特异性方式将分子附接到蛋白质或抗体的方法是本领域中已知的,并且根据本公开,本领域技术人员已知的位点特异性标记抗体的任何方法可用于本发明中。适用于本发明中的位点特异性修饰抗体的方法的示例包括但不限于掺入工程化半胱氨酸残基(例如, THIOMAB™)、使用非天然氨基酸或聚糖(例如, 硒代半胱氨酸、p-AcPhe、甲酰甘氨酸生成酶(FGE、SMARTag™)等)、以及酶学方法(例如, 使用糖基转移酶、内切糖苷酶、微生物或细菌转谷氨酰胺酶(MTG或BTG)、转肽酶A等)。根据优选的实施方案,修饰的多肽为通过用对抗体的Fc糖基化位点中

的核心GlcNAc残基之间的 β -1,4键联具有特异性的细菌内切糖苷酶(诸如GlycINATOR (Genovis))修剪抗体或其抗原结合片段来获得的抗体或其抗原结合片段,其使最内的GlcNAc完整保留在Fc上,从而允许叠氮基糖位点特异性掺入在该位点处。然后,可在糖转移酶诸如GalT半乳糖基转移酶或GalNAc转移酶的存在下,使修剪的抗体或其抗原结合片段与叠氮化物标记的糖(诸如UDP-N-叠氮基乙酰半乳糖胺(UDP-GalNAz)或UDP-6-叠氮基6-脱氧GalNAc)反应,从而获得修饰的抗体或其抗原结合片段。根据其他优选的实施方案,修饰的多肽为通过用酰胺酶使抗体或其抗原结合片段去糖基化来获得的抗体或其抗原结合片段。然后,可使所得去糖基化抗体或其抗原结合片段与叠氮基胺(优选地3-叠氮基丙胺、6-叠氮基己胺、或任何叠氮基-连接基-胺或任何叠氮基-烷基-胺,诸如叠氮基-聚乙二醇(PEG)-胺,例如0-(2-氨基乙基)-0'-(2-叠氮乙基)四乙二醇、0-(2-氨基乙基)-0'-(2-叠氮乙基)五乙二醇、0-(2-氨基乙基)-0'-(2-叠氮乙基)三乙二醇等)反应,或在微生物转谷氨酰胺酶的存在下反应,从而获得修饰的抗体或其抗原结合片段。

[0062] 如本文所用,术语“核酸适体”是指可以高亲和力特异性地结合其靶的单链寡核苷酸(单链DNA或RNA分子)。核酸适体可用作靶向各种有机和无机材料的分子。

[0063] 如本文所用,术语“小分子配体”是指低分子量有机化合物。如本文所用,小分子配体可指具有小于约1000道尔顿的尺寸的化合物,并且可在实验室中合成或天然存在。

[0064] 如本文所用,术语“点击反应配偶体”或“点击化学柄”是指可参与点击化学反应的反应物或反应性基团。点击反应配偶体可为很少存在于天然存在的生物分子中并且对生物分子呈化学惰性的部分,但是,例如当与叠氮化物反应性或炔烃反应性基团反应时,该反应可在生物相关条件下,例如在细胞培养条件下,诸如在不存在过量的热或苛刻的反应物的情况下有效地发生。一般来讲,点击化学反应需要至少两个包含可彼此反应的点击反应配偶体的分子。彼此反应的此类点击反应配偶体有时在本文中称为点击化学柄对或点击化学对。在一些实施方案中,点击反应配偶体是叠氮化物和应变炔烃,例如环辛炔或任何其他炔烃。在其他实施方案中,点击反应配偶体是反应性二烯和合适的四嗪亲双烯体。例如,反式环辛烯、降冰片烯或双环壬烯可与合适的四嗪亲双烯体配对作为点击反应对。在其他实施方案中,四唑可充当腈亚胺的潜在来源,其可在紫外光的存在下与未活化的烯烃配对以产生点击反应对,称为“光点击”反应对。在其他实施方案中,点击反应配偶体是半胱氨酸和马来酰亚胺。例如,来自肽(例如,GGGC)的半胱氨酸可与和螯合剂(例如,NOTA)缔合的马来酰亚胺反应。其他合适的点击化学柄是本领域技术人员已知的(参见,例如,Spicer等人, *Selective chemical protein modification. Nature Communications*. 2014;5:第4740页)。在其他实施方案中,点击反应配偶体是施陶丁格连接(Staudinger ligation)组分,诸如腈和叠氮化物。在其他实施方案中,点击反应配偶体是狄尔斯-阿尔德反应组分,诸如二烯,诸如四嗪,以及炔烃,诸如反式环辛烯(TCO)或降冰片烯。示例性点击反应配偶体描述于US20130266512和W02015073746中,两者中对于点击反应配偶体的相关描述均以引用方式并入本文。根据优选的实施方案,第一点击反应配偶体和第二点击反应配偶体中的一个包含炔烃基团,并且另一个点击反应配偶体包含叠氮化物。根据其他优选的实施方案,第一点击反应配偶体和第二点击反应配偶体中的一个包含烯烃基团,并且另一个点击反应配偶体包含二烯。

[0065] 如本文所用,术语“炔烃”、“炔烃基团”或“炔烃部分”是指包含碳-碳三键的官能

团。炔烃部分包括末端炔烃和环状炔烃,优选地与叠氮基团反应的末端炔烃和环状炔烃。末端炔烃具有至少一个结合到三键碳原子的氢原子。环状炔烃是包含一个或多个三键的环烷基环。环状炔烃的示例包括但不限于环辛炔和环辛炔衍生物,诸如双环壬炔(BCN)、二氟化环辛炔(DIFO)、二苯并环辛炔(DIBO)、酮基-DIBO、二芳基氮杂环辛酮(BARAC)、二苯并氮杂环辛炔(DIBAC)、二甲氧基氮杂环辛炔(DIMAC)、二苄基环辛炔(DBCO)、二氟苯并环辛炔(DIFBO)、单苯并环辛炔(MOBO)和四甲氧基DIBO(TMDIBO)。根据优选的实施方案,第一点击反应配偶体和第二点击反应配偶体中的一个包含环状炔烃,优选地DBCO。根据优选的实施方案,另一个点击反应配偶体包含叠氮化物,优选地NHS-叠氮化物。

[0066] 如本文所用,术语“二烯”是指具有两个碳-碳双键的化合物,其中这些双键在1,3-位置共轭。二烯的双键可为顺式或反式的。二烯的示例包括但不限于四嗪或四唑基团。

[0067] 如本文所用,术语“烯烃”、“烯烃基团”或“烯烃部分”是指包含碳-碳双键的不饱和烃分子。根据特定实施方案,烯烃可包含2至100个碳原子。烯烃的示例包括但不限于降冰片烯和反式环辛烯(TCO)。根据其他优选的实施方案,第一点击反应配偶体和第二点击反应配偶体中的一个包含烯烃基团,优选地降冰片烯或TCO。根据优选的实施方案,另一个点击反应配偶体包含二烯,优选地四嗪或四唑基团。

[0068] 如本文所用,术语“共价连接”意指多肽经由至少一个共价键联附接到第一点击反应配偶体,并且螯合剂经由至少一个共价键联附接到第二点击反应配偶体。键联可为直接的,即没有连接基,或间接的,即经由连接基。

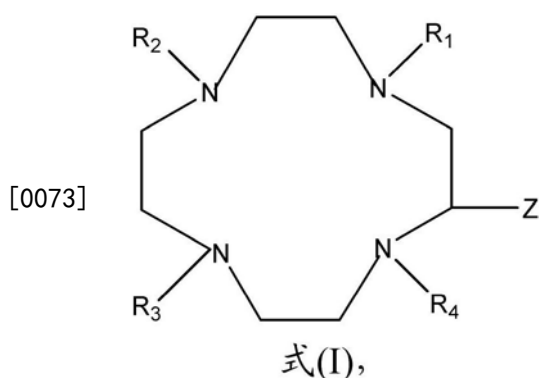
[0069] 如本文所用,术语“连接基”是指将多肽或螯合剂连接到点击反应配偶体的化学部分。根据本公开,本领域技术人员已知的任何合适的连接基可用于本发明中。连接基可为例如单个共价键、取代或未取代的烷基、取代或未取代的杂烷基部分、聚乙二醇(PEG)连接基、肽连接基、基于糖的连接基或可裂解的连接基,诸如二硫键联或蛋白酶裂解位点,诸如缬氨酸-瓜氨酸-PAB。

[0070] 如本文所用,术语“放射性金属离子(radiometal ion)”或“放射性金属离子(radioactive metal ion)”是指发射粒子和/或光子的元素的一种或多种同位素。根据本公开,本领域技术人员已知的任何放射性金属可用于本发明中。适用于本发明中的放射性金属的示例包括但不限于 ^{32}P 、 ^{47}Sc 、 ^{62}Cu 、 ^{64}Cu 、 ^{67}Cu 、 ^{67}Ga 、 ^{68}Ga 、 ^{77}As 、 ^{86}Y 、 ^{89}Zr 、 ^{89}Sr 、 ^{90}Y 、 ^{99}Tc 、 ^{105}Rh 、 ^{109}Pd 、 ^{111}Ag 、 ^{111}In 、 ^{117}Sn 、 ^{131}I 、 ^{153}Sm 、 ^{159}Gd 、 ^{165}Dy 、 ^{166}Ho 、 ^{169}Er 、 ^{177}Lu 、 ^{186}Re 、 ^{188}Re 、 ^{194}Ir 、 ^{198}Au 、 ^{199}Au 、 ^{211}At 、 ^{212}Pb 、 ^{212}Bi 、 ^{213}Bi 、 ^{223}Ra 、 ^{225}Ac 、 ^{227}Th 和 ^{255}Fm 。如本文所用,术语“诊断发射体”是指可用于诊断或成像应用中的放射性金属离子。诊断发射体的示例包括但不限于 γ 发射体,诸如 ^{62}Cu 、 ^{64}Cu 、 ^{67}Ga 、 ^{68}Ga 、 ^{86}Y 、 ^{89}Zr 和 ^{111}In 。如本文所用,术语“治疗发射体”是指可用于治疗应用中的放射性金属离子。治疗发射体的示例包括但不限于 β 或 α 发射体,诸如钷、镭、 ^{32}P 、 ^{47}Sc 、 ^{67}Cu 、 ^{77}As 、 ^{89}Sr 、 ^{90}Y 、 ^{99}Tc 、 ^{105}Rh 、 ^{109}Pd 、 ^{111}Ag 、 ^{131}I 、 ^{153}Sm 、 ^{159}Gd 、 ^{165}Dy 、 ^{166}Ho 、 ^{169}Er 、 ^{177}Lu 、 ^{186}Re 、 ^{188}Re 、 ^{194}Ir 、 ^{198}Au 、 ^{199}Au 、 ^{211}At 、 ^{212}Pb 、 ^{212}Bi 、 ^{213}Bi 、 ^{223}Ra 、 ^{225}Ac 、 ^{255}Fm 和 ^{227}Th 。根据优选的实施方案,放射性金属离子为 ^{225}Ac 。根据其他实施方案,多肽可用非金属放射性标记物进行标记以用于预靶向或诊疗应用中。适用于本发明中的非金属放射性标记物的示例包括但不限于 ^{125}I 和 ^{18}F 。

[0071] 本文所述的放射性复合物包含与螯合部分缔合的放射性金属离子。根据本发明的实施方案,螯合部分包含共价连接到点击反应配偶体的螯合剂,并且有时在本文中称为“双

官能螯合剂”。

[0072] 如本文所用,术语“螯合剂(chelant)”或“螯合剂(chelator)”是指放射性金属(诸如 ^{225}Ac)或金属可经由配位结合与其螯合的化合物。根据本公开,本领域技术人员已知的任何螯合剂可用于本发明中。在一个实施方案中,螯合剂包含大环。适用于本发明中的包含大环的螯合剂的示例包括但不限于去铁胺(DFO)、乙二胺四乙酸(EDTA)和二乙三胺五乙酸(DTPA)。在另一个实施方案中,螯合剂包含开链配体。适用于本发明中的包含开链配体的螯合剂的示例包括但不限于1,4,7,10-四氮杂环十二烷-N,N',N'',N'''-四乙酸(DOTA)、1,4,7,10,13,16-六氮杂环十六烷-N,N',N'',N''',N'''',N'''''-六乙酸(HEHA)、1,4,7,10,13-五氮杂环十五烷-N,N',N'',N''',N''''-五乙酸(PEPA)、Macropa (Thiele等人,An Eighteen-Membered Macrocyclic Ligand for Actinium-225 Targeted Alpha Therapy. Angew Chem Int Ed Engl. 2017年11月13日;56(46):第14712-14717页)、1,4,8,11-四氮杂环十四烷-1,4,8,11-四乙酸(TETA)、1,4,7,10-四氮杂环十二烷-1,4,7,10-四丙酸(DOTPA)、1,4,8,11-四氮杂环十四烷-1,4,8,11-四丙酸(TETPA)和1,4,7,10-四氮杂环十二烷-1,4,7,10-四亚甲基膦酸(DOTMP)。根据优选的实施方案,螯合剂包含式(I)的结构:



[0074] 其中R₁、R₂、R₃和R₄中的每一者独立地为CHQC₂X,其中

[0075] Q独立地为氢、C₁-C₄烷基或(C₁-C₂烷基)苯基,并且

[0076] X独立地为氢、苄基、C₁-C₄烷基;并且

[0077] Z为(CH₂)_nY,其中

[0078] n为1-10,并且

[0079] Y为共价连接到第二点击反应配偶体的亲电或亲核部分;

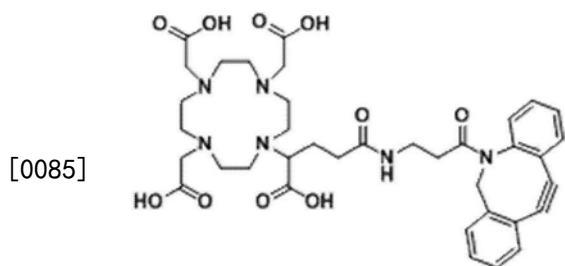
[0080] 另选地,Z为氢;并且

[0081] R₁、R₂、R₃和R₄中的每一者独立地为CHQC₂X,其中

[0082] Q独立地为氢、C₁-C₄烷基或(C₁-C₂烷基)苯基,并且

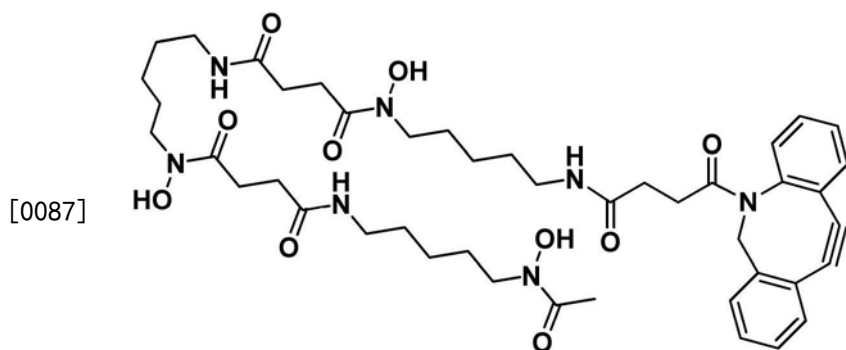
[0083] X独立地为氢、苄基、C₁-C₄烷基或者共价连接到第二点击反应配偶体的亲电或亲核部分。

[0084] 根据优选的实施方案,螯合部分包含式(II)的结构:



式(II)。

[0086] 根据优选的实施方案,整合部分包含式(III)的结构:



式(III)。

[0088] 在一个实施方案中,本发明涉及一种使用本发明的方法用两种或更多种放射性金属离子标记多肽的方法。例如,用两种放射性金属离子双重标记多肽的方法包括:

[0089] a. 提供修饰的多肽,该修饰的多肽包括共价连接到第一点击反应配偶体和第二点击反应配偶体的多肽;

[0090] b. 提供第一放射性复合物,该第一放射性复合物包含与整合部分缔合的第一放射性金属离子,其中整合部分包含共价连接到第三点击反应配偶体的整合剂;以及

[0091] c. 提供第二放射性复合物,该第二放射性复合物包含与整合部分缔合的第二放射性金属离子,其中整合部分包含共价连接到第四点击反应配偶体的整合剂;以及

[0092] d. 使修饰的多肽与第一放射性复合物和第二放射性复合物在允许第一点击反应配偶体与第三点击反应配偶体反应并且允许第二点击反应配偶体与第四点击反应配偶体反应的条件下接触,从而用第一放射性金属离子和第二放射性金属离子标记多肽。

[0093] 根据优选的实施方案,第一点击反应配偶体和第二点击反应配偶体中的一个包含炔基团,并且第一点击反应配偶体和第二点击反应配偶体中的另一个包含叠氮化物,并且其中第三点击反应配偶体和第四点击反应配偶体中的一个包含烯烃基团,并且第三点击反应配偶体和第四点击反应配偶体中的另一个包含二烯。

[0094] 根据优选的实施方案,第一放射性金属离子或第二放射性金属离子为诊断发射体,并且另一个为治疗发射体。根据其他优选的实施方案,第一放射性金属离子和第二放射性金属离子两者为治疗发射体。

[0095] 用于进行点击化学反应的条件是本领域中已知的,并且根据本公开,本领域技术人员已知的用于进行点击化学反应的任何条件可用于本发明中。条件的示例包括但不限于在4至10的pH和20℃至70℃的温度下以1:1至1000:1的比率温育修饰的多肽和放射性复合物。

[0096] 根据本公开,可使用本领域技术人员已知的方法分析本发明的点击放射性标记方法的产物。例如,LC/MS分析可用于测定螯合剂与标记的多肽的比率;分析性尺寸排阻色谱法可用于测定多肽和多肽缀合物的低聚状态;放射性化学收率可通过瞬时薄层色谱法(例如iTLC-SG)测定,并且放射性化学纯度可通过尺寸排阻HPLC测定。示例性方法在本文中描述于例如以下实施例中。

[0097] 药物组合物 and 治疗方法

[0098] 可将本发明的点击放射性标记方法修改为预靶向方法(Kraeber-Bodere, F.等人, A pretargeting system for tumor PET imaging and radioimmunotherapy. Front Pharmacol, 2015. 6: 第54页)。首先,给予叠氮基-mAb,使其与靶细胞结合,并允许其随时间推移从循环中清除,或用清除剂去除。随后,施用放射性复合物并使其经历与在靶位点处结合的叠氮基-mAb进行的SPAAC反应,而剩余的未结合的放射性复合物快速从循环中清除(Deal, K.A.等人, Improved in vivo stability of actinium-225 macrocyclic complexes. J Med Chem, 1999. 42 (15): 第2988-92页)。该预靶向技术提供了一种增强受治疗者中靶位点处的放射性金属离子定位的方法。

[0099] 因此,在另一个总体方面,本发明涉及一种药物组合物,该药物组合物包含药学上可接受的载体以及通过本发明的方法制备的放射性标记的多肽。

[0100] 如本文所用,术语“载体”是指任何赋形剂、稀释剂、填充剂、盐、缓冲剂、稳定剂、增溶剂、油、类脂、含脂质囊泡、微球体、脂质体包囊、或本领域公知的用于药物制剂中的其他材料。应当理解,载体、赋形剂或稀释剂的特征将取决于具体应用的施用途。如本文所用,术语“药学上可接受的载体”是指不干扰根据本发明的组合物的效果或根据本发明的组合物的生物活性的非毒性材料。根据本公开,根据特定实施方案,适用于基于抗体或基于放射性复合物的药物组合物中的任何药学上可接受的载体均可用于本发明中。

[0101] 根据特定实施方案,本文所述的组合物被配制或适于施用于受治疗者的预期途径。例如,本文所述的组合物可被配制或适于静脉内、皮下、肌内或瘤内施用。

[0102] 根据特定实施方案,修饰的多肽和放射性复合物可以相同或不同的组成施用。

[0103] 在另一个总体方面,本发明涉及一种在对其有需要的受治疗者中治疗肿瘤性疾病或障碍的方法,该方法包括向受治疗者施用本发明的药物组合物。

[0104] 根据特定实施方案,本发明的方法包括施用治疗有效剂量的本发明的药物组合物,其中组合物包含用于靶向与肿瘤性疾病或障碍相关的细胞的放射性标记的多肽,使得在靶向时,来自²²⁵Ac及其子代的α粒子被递送到靶向细胞并对其产生细胞毒性效应,从而治疗肿瘤性疾病或障碍。

[0105] 根据特定实施方案,以不同的组成施用治疗有效量的修饰的多肽和放射性复合物。

[0106] 如本文所用,术语“治疗有效量”是指在受治疗者中引起期望的生物学或药理学响应的活性成分或组分的量。治疗有效量可相对于指定目的根据经验并以常规方式进行确定。例如,可任选地采用体外测定来帮助确定最佳剂量范围。基于若干因素的考虑,包括要治疗或预防的疾病、所涉及的症状、患者的体重、患者的免疫状态以及技术人员已知的其他因素,本领域的技术人员可确定具体有效剂量的选择(例如,经由临床试验)。在制剂中待采用的精确剂量也将取决于施用途以及疾病的严重程度,并且应该根据医生的判断和每个

患者的情况来决定。有效剂量可通过源自体外或动物模型测试体系的剂量响应曲线来推导。

[0107] 如本文所用,术语“处理”和“治疗”(“treat”、“treating”和“treatment”)均旨在是指与其中放射性金属离子的施用将会有益的疾病、障碍或病症,诸如肿瘤性疾病或障碍有关的至少一种可测量物理参数的改善或逆转,其不一定是在受治疗者中可识别的,但可能是在受治疗者中可识别的。术语“处理”和“治疗”也可指导致消退,预防发展,或至少延缓疾病、障碍或病症的发展。在特定实施方案中,“处理”和“治疗”是指减轻与其中放射性金属离子的施用将会有益的疾病、障碍或病症,诸如肿瘤性疾病或障碍相关的一种或多种症状,预防该一种或多种症状的发展或发作,或者缩短该一种或多种症状的持续时间。在特定实施方案中,“处理”和“治疗”是指预防疾病、障碍或病症的复发。在特定实施方案中,“处理”和“治疗”是指患有疾病、障碍或病症的受治疗者的存活提高。在特定实施方案中,“处理”和“治疗”是指受治疗者中疾病、障碍或病症的消除。

[0108] 肿瘤性疾病或障碍的示例包括但不限于播散性癌症,实体肿瘤癌,肥大,冠状动脉疾病或血管闭塞性疾病,与受感染细胞、微生物或病毒相关的疾病或障碍,或者与炎性细胞相关的疾病或障碍,诸如类风湿性关节炎(RA)。

[0109] 如本文所用,术语“受治疗者”是指动物,并且优选地指哺乳动物。根据特定实施方案,受治疗者是哺乳动物,包括非灵长类(例如骆驼、驴、斑马、牛、猪、马、山羊、绵羊、猫、犬、大鼠、兔子、豚鼠、绒猴或小鼠)或灵长类(例如猴、黑猩猩或人)。在特定实施方案中,受治疗者是人。

[0110] 根据本公开,可使用修饰的多肽和放射性复合物的任何给药计划表。一般来讲,当以不同的组成施用修饰的多肽和放射性复合物时,可在施用修饰的抗体后的任何时间施用放射性复合物。

[0111] 根据特定实施方案,用于治疗肿瘤性疾病或障碍的组合物可与有效治疗相关肿瘤性疾病或障碍的其他药剂联合使用。

[0112] 如本文所用,在将两种或更多种疗法施用于受治疗者的上下文中,术语“联合”是指使用多于一种疗法。术语“联合”的使用并不限制疗法施用于受治疗者的次序。例如,第一疗法(例如,本文所述的组合物)可在第二疗法施用于受治疗者之前(例如5分钟、15分钟、30分钟、45分钟、1小时、2小时、4小时、6小时、12小时、16小时、24小时、48小时、72小时、96小时、1周、2周、3周、4周、5周、6周、8周、或12周以前),与此同时,或之后(例如5分钟、15分钟、30分钟、45分钟、1小时、2小时、4小时、6小时、12小时、16小时、24小时、48小时、72小时、96小时、1周、2周、3周、4周、5周、6周、8周、或12周以后)施用。

[0113] 在另一个总体方面,本发明涉及一种诊疗剂,该诊疗剂包含药学上可接受的载体以及通过本发明的方法制备的放射性标记的抗体,其中保留了放射性标记的抗体的免疫学特性。

[0114] 如本文所用,术语“诊疗”是指提供诊断功能和治疗功能中的任一种的能力。在一个实施方案中,诊疗剂提供诊断功能和治疗功能两者。在另一个实施方案中,诊疗剂是无诊断功能的活性药剂。在另一个实施方案中,诊疗剂是可用于诊断但不具有治疗功能的药剂。

[0115] 根据优选的实施方案,放射性金属离子为诊断发射体,优选地⁸⁹Zr。根据其他优选的实施方案,放射性金属离子为治疗发射体,优选地²²⁵Ac。根据优选的实施方案,诊疗剂用

于向对其有需要的受治疗者提供诊断功能和治疗功能两者。

[0116] 组合和试剂盒

[0117] 本文提供了一种组合,该组合包括:

[0118] a. 修饰的多肽,该修饰的多肽包括共价连接到第一点击反应配偶体的多肽;以及

[0119] b. 放射性复合物,该放射性复合物包含与螯合部分缔合的放射性金属离子,其中螯合部分包含共价连接到第二点击反应配偶体的螯合剂;

[0120] 其中组合将用于用放射性金属离子标记多肽。

[0121] 根据特定实施方案,本发明的组合是用于用放射性金属离子标记多肽的反应混合物。根据其他实施方案,组合是用于在体外或体内产生放射性标记的多肽的包装或试剂盒。该组合可以任选地随附呈管理药物或生物产品的制造、使用或销售的政府部门规定的形式的通知或说明书,该通知或说明书反映了管理部门批准用于人类施用的制造、使用或销售。本文所涵盖的组合可用于用放射性金属离子标记多肽或者在对其有需要的受治疗者中治疗肿瘤性疾病或障碍的上述方法中。

[0122] 实施方案

[0123] 本发明还提供了以下非限制性实施方案。

[0124] 实施方案1是一种用放射性金属离子标记多肽的方法,所述方法包括:

[0125] a. 提供修饰的多肽,所述修饰的多肽包括共价连接到第一点击反应配偶体的多肽;

[0126] b. 提供放射性复合物,所述放射性复合物包含与螯合部分缔合的所述放射性金属离子,其中所述螯合部分包含共价连接到第二点击反应配偶体的螯合剂;以及

[0127] c. 使所述修饰的多肽与所述放射性复合物在允许所述第一点击反应配偶体与所述第二点击反应配偶体反应的条件下接触,从而用所述放射性金属离子标记所述多肽。

[0128] 实施方案1a是根据实施方案1所述的方法,其中所述螯合剂包含大环。

[0129] 实施方案1b是根据实施方案1所述的方法,其中所述螯合剂包含开链配体。

[0130] 实施方案2是根据实施方案1所述的方法,其中所述第一点击反应配偶体和所述第二点击反应配偶体中的一个包含炔烃基团,并且另一个点击反应配偶体包含叠氮化物。

[0131] 实施方案3是根据实施方案2所述的方法,其中所述第一点击反应配偶体包含叠氮基团,并且所述第二点击反应配偶体包含炔烃基团。

[0132] 实施方案3a是根据实施方案2或3所述的方法,其中所述炔烃基团包括末端炔烃。

[0133] 实施方案3b是根据实施方案2或3所述的方法,其中所述炔烃基团包括环状炔烃,优选地环辛炔或环辛炔衍生物。

[0134] 实施方案3c是根据实施方案3b所述的方法,其中所述炔烃基团包括双环壬炔(BCN)。

[0135] 实施方案3d是根据实施方案3b所述的方法,其中所述炔烃基团包括二氟化环辛炔(DIFO)。

[0136] 实施方案3e是根据实施方案3b所述的方法,其中所述炔烃基团包括二苯并环辛炔(DIBO)。

[0137] 实施方案3f是根据实施方案3b所述的方法,其中所述炔烃基团包括二芳基氮杂环辛酮(BARAC)。

[0138] 实施方案3g是根据实施方案3b所述的方法,其中所述炔烃基团包括二苯并氮杂环辛炔(DIBAC)。

[0139] 实施方案3h是根据实施方案3b所述的方法,其中所述炔烃基团包括二甲氧基氮杂环辛炔(DIMAC)。

[0140] 实施方案3i是根据实施方案3b所述的方法,其中所述炔烃基团包括二苄基环辛炔(DBCO)。

[0141] 实施方案3j是根据实施方案3b所述的方法,其中所述炔烃基团包括二氟苯并环辛炔(DIFBO)。

[0142] 实施方案3k是根据实施方案3b所述的方法,其中所述炔烃基团包括单苯并环辛炔(MOBO)。

[0143] 实施方案3l是根据实施方案3b所述的方法,其中所述炔烃基团包括四甲氧基DIBO(TMDIBO)。

[0144] 实施方案3m是根据实施方案2至3l中任一项所述的方法,其中所述叠氮基团包括NHS-叠氮化物。

[0145] 实施方案4是根据实施方案1所述的方法,其中所述第一点击反应配偶体和所述第二点击反应配偶体中的一个包含烯炔基团,并且另一个点击反应配偶体包含二烯。

[0146] 实施方案4a是根据实施方案4所述的方法,其中所述二烯包含四嗪或四唑基团。

[0147] 实施方案4b是根据实施方案4或4a所述的方法,其中所述烯炔基团包括降冰片烯。

[0148] 实施方案4c是根据实施方案4或4a所述的方法,其中所述烯炔基团包括反式环辛烯(TCO)。

[0149] 实施方案5是根据实施方案1至4c中任一项所述的方法,其中所述多肽为抗体或其抗原结合片段。

[0150] 实施方案6是根据实施方案5所述的方法,其中所述抗体为单克隆抗体或其抗原结合片段。

[0151] 实施方案6a是根据实施方案1至6中任一项所述的方法,其中所述修饰的多肽通过将一或多个叠氮基团随机缀合到所述多肽来获得。

[0152] 实施方案6b是根据实施方案1至6中任一项所述的方法,其中所述修饰的多肽为通过位点特异性掺入所述第一点击反应配偶体来获得的修饰的抗体或其抗原结合片段。

[0153] 实施方案6c是根据实施方案6b所述的方法,其中所述修饰的抗体或其抗原结合片段通过用对所述抗体的一个或多个Fc糖基化位点中的一个或多个核心GlcNac残基之间的 β -1,4键联具有特异性的细菌内切糖苷酶修剪所述抗体或其抗原结合片段,以获得修剪的抗体或其抗原结合片段,以及在糖转移酶,优选地GalT半乳糖基转移酶的存在下使所述修剪的抗体或其抗原结合片段与叠氮糖,优选地UDP-GalNAz叠氮基糖底物反应来获得。

[0154] 实施方案6d是根据实施方案6b所述的方法,其中所述修饰的抗体或其抗原结合片段通过用酰胺酶使所述抗体或其抗原结合片段去糖基化,以获得去糖基化的抗体或其抗原结合片段,以及在微生物转谷氨酰胺酶的存在下使所述去糖基化的抗体或其抗原结合片段与叠氮基胺,优选地3-叠氮基丙胺反应来获得。

[0155] 实施方案6e是根据实施方案6至6d中任一项所述的方法,其中所述抗体为结合人前列腺特异性膜抗原(PSMA)的抗体或其抗原结合片段,优选地所述抗体包含SEQ ID NO:3

的HC CDR1序列、SEQ ID NO:4的HC CDR2序列、SEQ ID NO:5的HC CDR3序列、SEQ ID NO:6的轻链(LC)CDR1序列、SEQ ID NO:7的LC CDR2序列和SEQ ID NO:8的LC CDR3序列。

[0156] 实施方案6f是根据实施方案6e所述的方法,其中所述抗体包含SEQ ID NO:9的HC序列和SEQ ID NO:10的LC序列。

[0157] 实施方案7是根据实施方案1至6d中任一项所述的方法,其中所述放射性金属离子为 ^{32}P 、 ^{47}Sc 、 ^{67}Cu 、 ^{77}As 、 ^{89}Sr 、 ^{90}Y 、 ^{99}Tc 、 ^{105}Rh 、 ^{109}Pd 、 ^{111}Ag 、 ^{131}I 、 ^{153}Sm 、 ^{159}Gd 、 ^{165}Dy 、 ^{166}Ho 、 ^{169}Er 、 ^{177}Lu 、 ^{186}Re 、 ^{188}Re 、 ^{194}Ir 、 ^{198}Au 、 ^{199}Au 、 ^{211}At 、 ^{212}Pb 、 ^{212}Bi 、 ^{213}Bi 、 ^{223}Ra 、 ^{225}Ac 、 ^{255}Fm 、 ^{227}Th 、 ^{62}Cu 、 ^{64}Cu 、 ^{67}Ga 、 ^{68}Ga 、 ^{86}Y 、 ^{89}Zr 或 ^{111}In 。

[0158] 实施方案7a是根据实施方案1至6d中任一项所述的方法,其中所述放射性金属离子为 ^{225}Ac 。

[0159] 实施方案7b是根据实施方案1至6d中任一项所述的方法,其中所述放射性金属离子为 ^{111}In 。

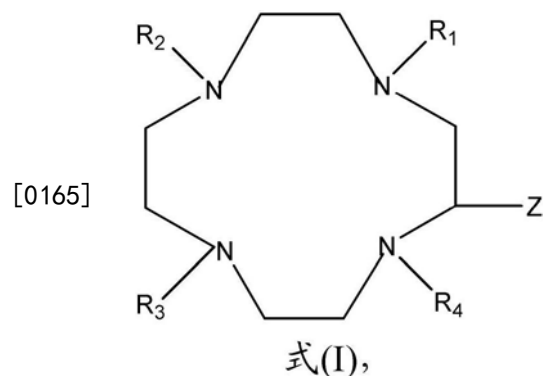
[0160] 实施方案7c是根据实施方案1至6d中任一项所述的方法,其中所述放射性金属离子为 ^{89}Zr 。

[0161] 实施方案8是根据实施方案1至7c中任一项所述的方法,其中所述螯合部分经由连接基共价连接到所述第二点击反应配偶体。

[0162] 实施方案9是根据实施方案1至8中任一项所述的方法,还包括使位于所述多肽上或引入到所述多肽的侧链,优选地赖氨酸的氨基侧链上的亲电体与共价连接到所述第一点击反应配偶体的巯基基团反应,以获得所述修饰的多肽,优选地NHS-叠氮化物。

[0163] 实施方案10是根据实施方案1至9中任一项所述的方法,其中所述修饰的多肽包括直接或经由连接基共价连接到叠氮、四嗪或四唑基团的多肽。

[0164] 实施方案11是根据实施方案1至10中任一项所述的方法,其中所述螯合剂包含大环,优选地式(I)的结构:



[0166] 其中 R_1 、 R_2 、 R_3 和 R_4 中的每一者独立地为 $\text{CH}_2\text{QCO}_2\text{X}$,其中

[0167] Q独立地为氢、 C_1 - C_4 烷基或(C_1 - C_2 烷基)苯基,并且

[0168] X独立地为氢、苄基、 C_1 - C_4 烷基;并且

[0169] Z为 $(\text{CH}_2)_n\text{Y}$,其中

[0170] n为1-10,并且

[0171] Y为共价连接到所述第二点击反应配偶体的亲电或亲核部分;

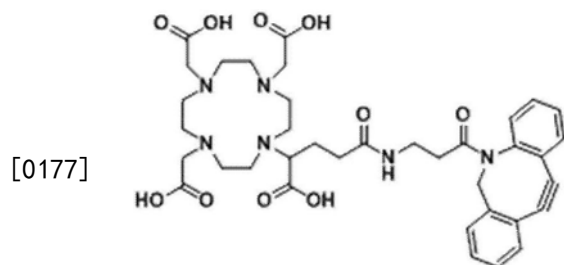
[0172] 另选地,Z为氢;并且

[0173] R_1 、 R_2 、 R_3 和 R_4 中的每一者独立地为 $\text{CH}_2\text{QCO}_2\text{X}$,其中

[0174] Q独立地为氢、C₁-C₄烷基或(C₁-C₂烷基)苯基,并且

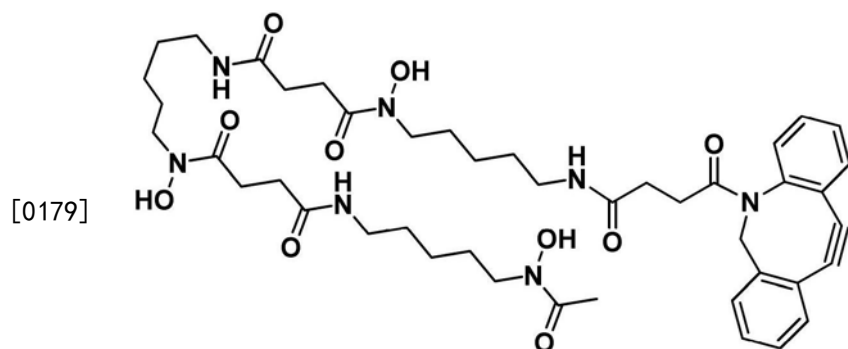
[0175] X独立地为氢、苄基、C₁-C₄烷基或者共价连接到所述第二点击反应配偶体的亲电或亲核部分。

[0176] 实施方案12是根据实施方案1至11中任一项所述的方法,其中所述螯合部分包含式(II)的结构:



式(II)。

[0178] 实施方案12a是根据实施方案1至11中任一项所述的方法,其中所述螯合部分包含具有开链配体的螯合剂,优选地具有式(III)的结构的螯合部分:



式(III)。

[0180] 实施方案12b是根据实施方案1至10中任一项所述的方法,其中所述螯合部分包含选自以下项组成的组的螯合剂:1,4,7,10-四氮杂环十二烷-N,N',N'',N'''-四乙酸(DOTA)、去铁胺(DFO)、1,4,7,10,13,16-六氮杂环十六烷-N,N',N'',N''',N''',N''''-六乙酸(HEHA)、1,4,7,10,13-五氮杂环十五烷-N,N',N'',N''',N''''-五乙酸(PEPA)、乙二胺四乙酸(EDTA)、二乙三胺五乙酸(DTPA)、Macropa(Thiele等人,An Eighteen-Membered Macrocyclic Ligand for Actinium-225 Targeted Alpha Therapy. Angew Chem Int Ed Engl. 2017年11月13日;56(46):第14712-14717页)、1,4,8,11-四氮杂环十四烷-1,4,8,11-四乙酸(TETA)、1,4,7,10-四氮杂环十二烷-1,4,7,10-四丙酸(DOTPA)、1,4,8,11-四氮杂环十四烷-1,4,8,11-四丙酸(TETPA)和1,4,7,10-四氮杂环十二烷-1,4,7,10-四亚甲基膦酸(DOTMP)。

[0181] 实施方案12c是根据实施方案12b所述的方法,其中所述螯合剂包括1,4,7,10-四氮杂环十二烷-N,N',N'',N'''-四乙酸(DOTA)。

[0182] 实施方案12d是根据实施方案12b所述的方法,其中所述螯合剂包括去铁胺(DFO)。

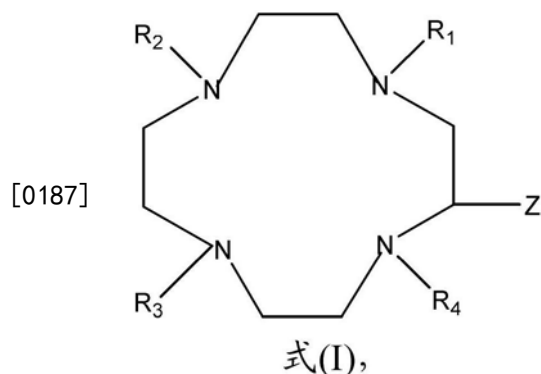
[0183] 实施方案13是一种用放射性金属离子,优选地²²⁵Ac、¹¹¹In或⁸⁹Zr标记多肽,优选地抗体或其抗原结合片段的方法,所述方法包括:

[0184] a. 提供修饰的多肽,优选地修饰的抗体或其抗原结合片段,所述修饰的抗体或其

抗原结合片段包含共价连接到叠氮、四嗪或四唑基团的多肽或抗体或其抗原结合片段；

[0185] b. 提供放射性复合物，所述放射性复合物包含与螯合部分缔合的所述放射性金属离子，优选地²²⁵Ac、¹¹¹In或⁸⁹Zr，其中所述螯合部分包含共价连接到炔烃或烯炔基团的螯合剂；以及

[0186] c. 使所述修饰的多肽或抗体或其抗原结合片段与所述放射性复合物在允许所述叠氮、四嗪或四唑基团与所述炔烃或所述烯炔基团反应的条件下接触，从而用所述放射性金属离子，优选地²²⁵Ac、¹¹¹In或⁸⁹Zr标记所述多肽或抗体或其抗原结合片段，其中所述螯合剂包含式(I)的结构：



[0188] 其中R₁、R₂、R₃和R₄中的每一者独立地为CHQC₂X，其中

[0189] Q独立地为氢、C₁-C₄烷基或(C₁-C₂烷基)苯基，并且

[0190] X独立地为氢、苄基、C₁-C₄烷基；并且

[0191] Z为(CH₂)_nY，其中

[0192] n为1-10，并且

[0193] Y为共价连接到所述炔烃基团的亲电或亲核部分；

[0194] 另选地，Z为氢；并且

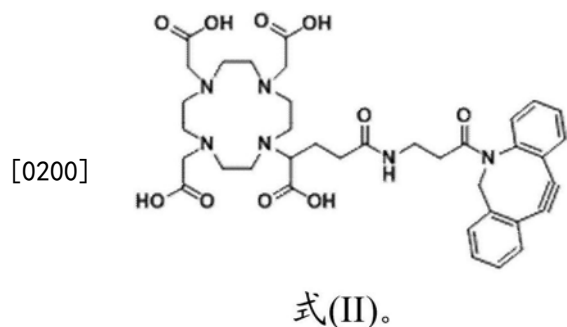
[0195] R₁、R₂、R₃和R₄中的每一者独立地为CHQC₂X，其中

[0196] Q独立地为氢、C₁-C₄烷基或(C₁-C₂烷基)苯基，并且

[0197] X独立地为氢、苄基、C₁-C₄烷基或者共价连接到所述炔烃基团的亲电或亲核部分。

[0198] 实施方案13a是根据实施方案13所述的方法，其中所述螯合剂包括1,4,7,10-四氮杂环十二烷-N,N',N'',N'''-四乙酸(DOTA)。

[0199] 实施方案13b是根据实施方案13所述的方法，其中所述螯合部分包含式(II)的结构：



[0201] 实施方案13d是一种用放射性金属离子，优选地²²⁵Ac、¹¹¹In或⁸⁹Zr标记多肽，优选地抗体或其抗原结合片段的方法，所述方法包括：

[0202] a. 提供修饰的多肽, 优选地修饰的抗体或其抗原结合片段, 所述修饰的抗体或其抗原结合片段包含共价连接到叠氮、四嗪或四唑基团的多肽或抗体或其抗原结合片段;

[0203] b. 提供放射性复合物, 所述放射性复合物包含与螯合部分缔合的所述放射性金属离子, 优选地 ^{225}Ac 、 ^{111}In 或 ^{89}Zr , 其中所述螯合部分包含共价连接到炔烃或烯炔基团的螯合剂; 以及

[0204] c. 使所述修饰的多肽或抗体或其抗原结合片段与所述放射性复合物在允许所述叠氮、四嗪或四唑基团与所述炔烃或所述烯炔基团反应的条件下接触, 从而用所述放射性金属离子, 优选地 ^{225}Ac 、 ^{111}In 或 ^{89}Zr 标记所述多肽或抗体或其抗原结合片段,

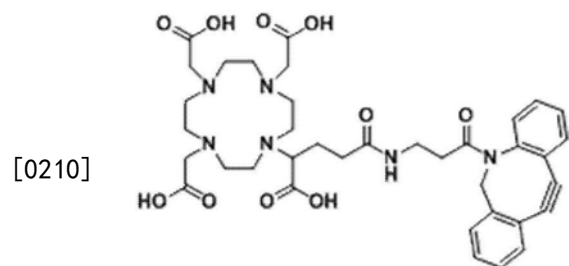
[0205] 其中所述螯合剂包含开链配体, 优选地去铁胺 (DFO)。

[0206] 实施方案14是根据实施方案13至13c中任一项所述的方法, 其中所述螯合剂经由连接基共价连接到所述炔烃或烯炔基团。

[0207] 实施方案15是根据实施方案13至14中任一项所述的方法, 还包括使位于所述多肽, 优选地所述抗体或其抗原结合片段上或引入到所述多肽的侧链, 优选地赖氨酸的氨基侧链上的亲电体与共价连接到叠氮化物, 优选地NHS-叠氮化物的巯基基团反应, 以获得所述修饰的多肽或抗体或其抗原结合片段。

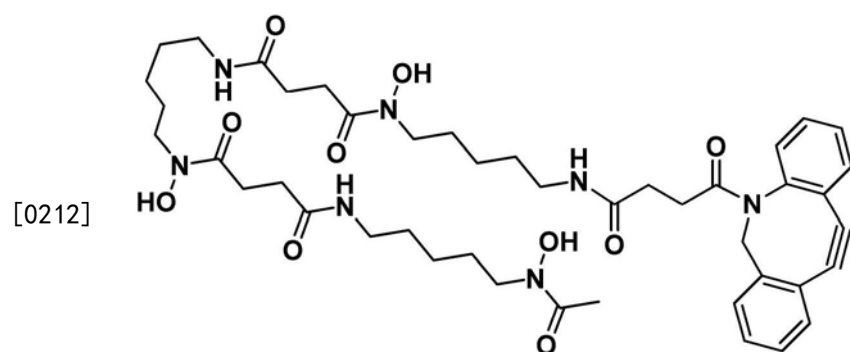
[0208] 实施方案16是根据实施方案13至15中任一项所述的方法, 其中所述多肽, 优选地抗体或其抗原结合片段经由连接基共价连接到所述叠氮化物。

[0209] 实施方案17是根据实施方案13所述的方法, 其中所述多肽为抗体或其抗原结合片段, 所述放射性金属离子为 ^{225}Ac 、 ^{111}In 或 ^{89}Zr , 并且所述螯合部分包含式(II)的结构:



式(II)。

[0211] 实施方案17a是根据实施方案13c所述的方法, 其中所述多肽为抗体或其抗原结合片段, 所述放射性金属离子为 ^{225}Ac 、 ^{111}In 或 ^{89}Zr , 并且所述螯合部分包含式(III)的结构:



式(III)。

[0213] 实施方案17b是根据实施方案13至17a中任一项所述的方法, 其中所述多肽为结合人前列腺特异性膜抗原 (PSMA) 或其抗原结合片段的抗体, 优选地所述抗体包含SEQ ID NO:

3的HC CDR1序列、SEQ ID NO:4的HC CDR2序列、SEQ ID NO:5的HC CDR3序列、SEQ ID NO:6的轻链(LC)CDR1序列、SEQ ID NO:7的LC CDR2序列和SEQ ID NO:8的LC CDR3序列。

[0214] 实施方案17c是根据实施方案17b所述的方法,其中所述抗体包含SEQ ID NO:9的HC序列和SEQ ID NO:10的LC序列。

[0215] 实施方案18是一种用两种放射性金属离子双重标记多肽的方法,所述方法包括:

[0216] a.提供修饰的多肽,所述修饰的多肽包括共价连接到第一点击反应配偶体和第二点击反应配偶体的多肽;

[0217] b.提供第一放射性复合物,所述第一放射性复合物包含与螯合部分缔合的第一放射性金属离子,其中所述螯合部分包含共价连接到第三点击反应配偶体的螯合剂;以及

[0218] c.提供第二放射性复合物,所述第二放射性复合物包含与螯合部分缔合的第二放射性金属离子,其中所述螯合部分包含共价连接到第四点击反应配偶体的螯合剂;以及

[0219] d.使所述修饰的多肽与所述第一放射性复合物和所述第二放射性复合物在允许所述第一点击反应配偶体与所述第三点击反应配偶体反应并且允许所述第二点击反应配偶体与所述第四点击反应配偶体反应的条件接触,从而用所述第一放射性金属离子和所述第二放射性金属离子标记所述多肽。

[0220] 实施方案19是根据实施方案18所述的方法,其中所述第一点击反应配偶体和所述第二点击反应配偶体中的一个包含炔烃基团,并且所述第一点击反应配偶体和所述第二点击反应配偶体中的另一个包含叠氮化物,并且其中所述第三点击反应配偶体和所述第四点击反应配偶体中的一个包含烯炔基团,并且所述第三点击反应配偶体和所述第四点击反应配偶体中的另一个包含二烯。

[0221] 实施方案20是根据实施方案18或19所述的方法,其中所述第一放射性金属离子或所述第二放射性金属离子为诊断发射体,并且另一个为治疗发射体。

[0222] 实施方案21是根据实施方案18或19所述的方法,其中所述第一放射性金属离子和所述第二放射性金属离子两者为治疗发射体。

[0223] 实施方案21a是根据实施方案20或21所述的方法,其中所述诊断发射体为 ^{62}Cu 、 ^{64}Cu 、 ^{67}Ga 、 ^{68}Ga 、 ^{86}Y 、 ^{89}Zr 或 ^{111}In 。

[0224] 实施方案21b是根据实施方案20至21a中任一项所述的方法,其中所述治疗发射体为 ^{32}P 、 ^{47}Sc 、 ^{67}Cu 、 ^{77}As 、 ^{89}Sr 、 ^{90}Y 、 ^{99}Tc 、 ^{105}Rh 、 ^{109}Pd 、 ^{111}Ag 、 ^{131}I 、 ^{153}Sm 、 ^{159}Gd 、 ^{165}Dy 、 ^{166}Ho 、 ^{169}Er 、 ^{177}Lu 、 ^{186}Re 、 ^{188}Re 、 ^{194}Ir 、 ^{198}Au 、 ^{199}Au 、 ^{211}At 、 ^{212}Pb 、 ^{212}Bi 、 ^{213}Bi 、 ^{223}Ra 、 ^{225}Ac 、 ^{255}Fm 或 ^{227}Th 。

[0225] 实施方案22是一种药物组合物,包含药学上可接受的载体以及通过根据实施方案1至21b中任一项所述的方法制备的放射性标记的多肽。

[0226] 实施方案23是一种在对其有需要的受治疗者中治疗或诊断疾病或障碍,特别是肿瘤性疾病或障碍的方法,包括向所述受治疗者施用根据实施方案22所述的药物组合物。

[0227] 实施方案24是根据实施方案23所述的方法,其中所述药物组合物包括待依次施用的两种组合物,第一组合物包含所述修饰的多肽,并且第二组合物包含一种或多种放射性复合物。

[0228] 实施方案25是一种诊疗剂,包含药学上可接受的载体以及通过根据实施方案1至21b中任一项所述的方法制备的放射性标记的抗体,其中保留了所述放射性标记的抗体的免疫学特性。

[0229] 实施方案26是根据实施方案25所述的诊疗剂,其中所述放射性金属离子为诊断发射体,优选地 ^{89}Zr 。

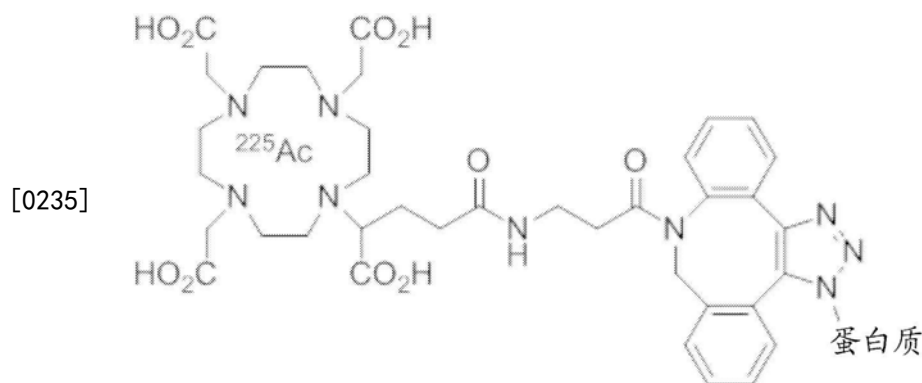
[0230] 实施方案27是根据实施方案25所述的诊疗剂,其中所述放射性金属离子为治疗发射体,优选地 ^{225}Ac 。

[0231] 实施方案27a是根据实施方案25所述的诊疗剂,其中所述放射性金属离子为 ^{111}In 。

[0232] 实施方案27b是根据实施方案25至27a中任一项所述的诊疗剂,其中所述多肽为结合人前列腺特异性膜抗原(PSMA)或其抗原结合片段的抗体,优选地所述抗体包含SEQ ID NO:3的HC CDR1序列、SEQ ID NO:4的HC CDR2序列、SEQ ID NO:5的HC CDR3序列、SEQ ID NO:6的轻链(LC)CDR1序列、SEQ ID NO:7的LC CDR2序列和SEQ ID NO:8的LC CDR3序列。

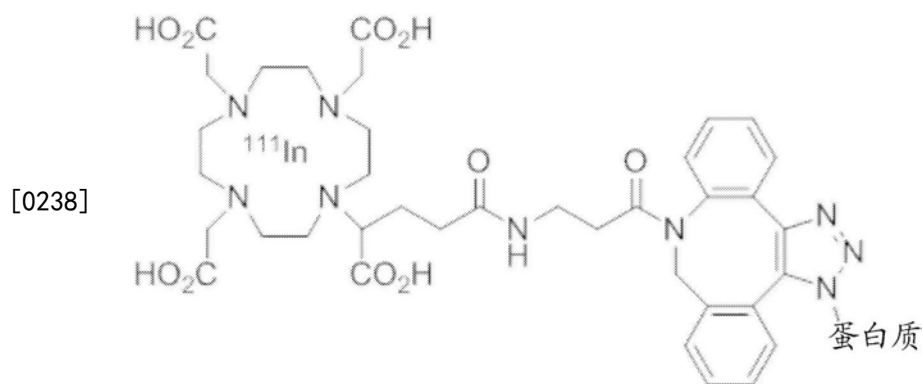
[0233] 实施方案27c是根据实施方案27b所述的诊疗剂,其中所述抗体包含SEQ ID NO:9的HC序列和SEQ ID NO:10的LC序列。

[0234] 实施方案27d是根据实施方案25至27c中任一项所述的诊疗剂,其中所述放射性标记的抗体具有式(IV)的式:



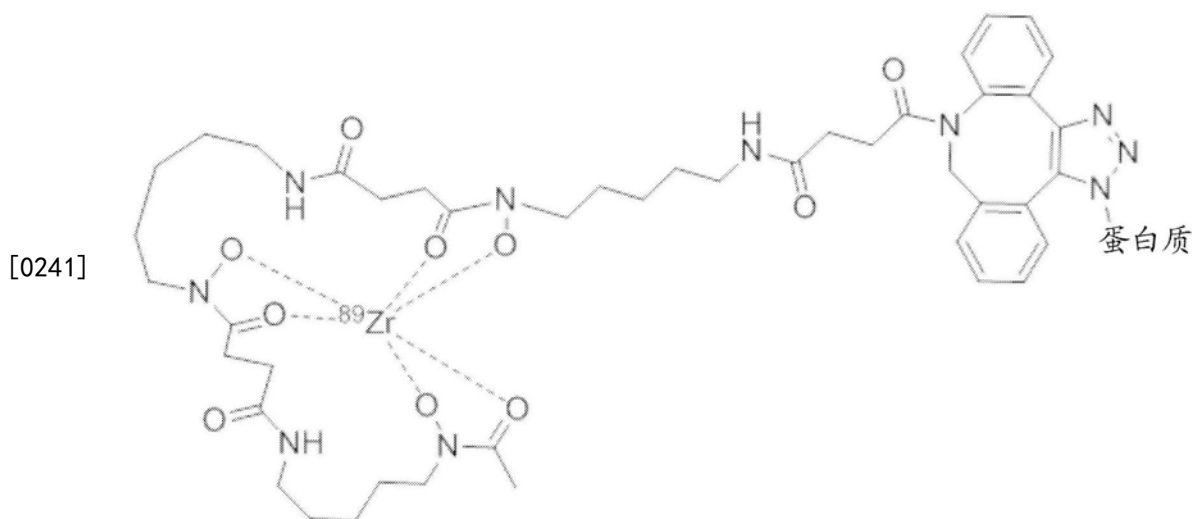
[0236] 式(IV) (DOTA-Ac-DBCO-蛋白质),另选地,所述 ^{225}Ac 被另一种放射性金属离子取代,所述另一种放射性金属离子诸如 ^{32}P 、 ^{47}Sc 、 ^{67}Cu 、 ^{77}As 、 ^{89}Sr 、 ^{90}Y 、 ^{99}Tc 、 ^{105}Rh 、 ^{109}Pd 、 ^{111}Ag 、 ^{131}I 、 ^{153}Sm 、 ^{159}Gd 、 ^{165}Dy 、 ^{166}Ho 、 ^{169}Er 、 ^{177}Lu 、 ^{186}Re 、 ^{188}Re 、 ^{194}Ir 、 ^{198}Au 、 ^{199}Au 、 ^{211}At 、 ^{212}Pb 、 ^{212}Bi 、 ^{213}Bi 、 ^{223}Ra 、 ^{255}Fm 、 ^{227}Th 、 ^{62}Cu 、 ^{64}Cu 、 ^{67}Ga 、 ^{68}Ga 、 ^{86}Y 、 ^{89}Zr 或 ^{111}In 。

[0237] 实施方案27e是根据实施方案25至27c中任一项所述的诊疗剂,其中所述放射性标记的抗体具有式(V)的式:



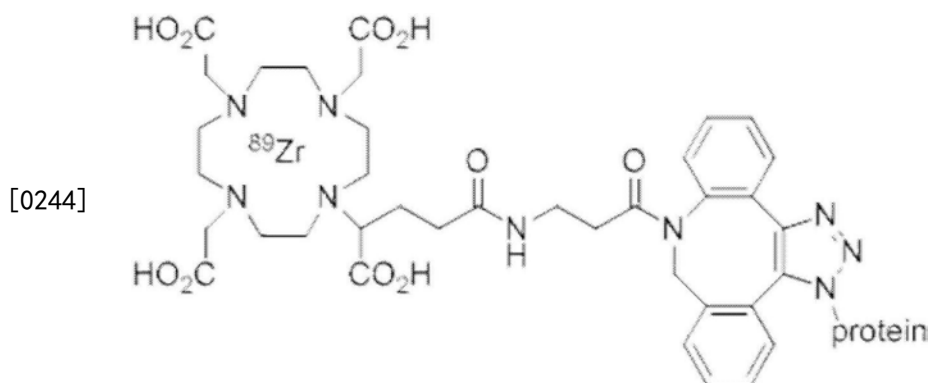
[0239] 式(V) (DOTA-In-DBCO-蛋白质)。

[0240] 实施方案27f是根据实施方案25至27c中任一项所述的诊疗剂,其中所述放射性标记的抗体具有式(VI)的式:



[0242] 式(VI) (DF0-Zr-DBCO-蛋白质), 另选地, 所述 ^{89}Zr 被另一种放射性金属离子取代, 所述另一种放射性金属离子诸如 ^{32}P 、 ^{47}Sc 、 ^{67}Cu 、 ^{77}As 、 ^{89}Sr 、 ^{90}Y 、 ^{99}Tc 、 ^{105}Rh 、 ^{109}Pd 、 ^{111}Ag 、 ^{131}I 、 ^{153}Sm 、 ^{159}Gd 、 ^{165}Dy 、 ^{166}Ho 、 ^{169}Er 、 ^{177}Lu 、 ^{186}Re 、 ^{188}Re 、 ^{194}Ir 、 ^{198}Au 、 ^{199}Au 、 ^{211}At 、 ^{212}Pb 、 ^{212}Bi 、 ^{213}Bi 、 ^{223}Ra 、 ^{225}Ac 、 ^{255}Fm 、 ^{227}Th 、 ^{62}Cu 、 ^{64}Cu 、 ^{67}Ga 、 ^{68}Ga 、 ^{86}Y 或 ^{111}In 。

[0243] 实施方案27g是根据实施方案25至27c中任一项所述的诊疗剂, 其中所述放射性标记的抗体具有式(VII)的式:



[0245] 式(VII) (DOTA-Zr-DBCO-蛋白质)。

[0246] 实施方案27h是根据实施方案25至27g中任一项所述的诊疗剂, 其中所述放射性标记的抗体具有小于3的螯合剂: 抗体比率 (CAR)。

[0247] 实施方案27i是根据实施方案25至27h中任一项所述的诊疗剂, 其中所述放射性标记的抗体具有2的螯合剂: 抗体比率 (CAR)。

[0248] 实施方案28是一种组合, 优选地试剂盒, 包括:

[0249] a. 修饰的多肽, 所述修饰的多肽包括共价连接到第一点击反应配偶体的多肽; 以及

[0250] b. 放射性复合物, 所述放射性复合物包含与螯合部分缔合的放射性金属离子, 其中所述螯合部分包含共价连接到第二点击反应配偶体的螯合剂;

[0251] 其中所述组合将用于用所述放射性金属离子标记所述多肽。

[0252] 实施方案28a是根据实施方案28所述的组合或试剂盒, 其中所述螯合剂包含大环。

[0253] 实施方案28b是根据实施方案28所述的组合或试剂盒, 其中所述螯合剂包含开链配体。

[0254] 实施方案29是根据实施方案28所述的组合或试剂盒,所述组合或试剂盒将用于经由所述第一点击反应配偶体与所述第二点击反应配偶体之间的体外反应用所述放射性金属离子标记所述多肽。

[0255] 实施方案30是根据实施方案28所述的组合或试剂盒,所述组合或试剂盒将用于经由所述第一点击反应配偶体与所述第二点击反应配偶体之间的体内反应用所述放射性金属离子标记所述多肽。

[0256] 实施方案31是一种组合物,所述组合物包含修饰的多肽,所述修饰的多肽包括共价连接到第一点击反应配偶体的多肽。

[0257] 实施方案32是一种组合物,所述组合物包含放射性复合物,所述放射性复合物包含与螯合部分缔合的放射性金属离子,其中所述螯合部分包含共价连接到第二点击反应配偶体的螯合剂。

[0258] 实施方案32a是根据实施方案32所述的组合物,其中所述螯合剂包含大环。

[0259] 实施方案32b是根据实施方案32所述的组合物,其中所述螯合剂包含开链配体。

[0260] 实施方案33是根据实施方案28至30中任一项所述的组合或试剂盒或者根据实施方案31或32所述的组合物,其中所述多肽为抗体或其抗原结合片段。

[0261] 实施方案33a是根据实施方案33所述的组合或试剂盒或组合物,其中所述抗体能够结合人前列腺特异性膜抗原(PSMA)或其抗原结合片段,优选地所述抗体包含SEQ ID NO:3的HC CDR1序列、SEQ ID NO:4的HC CDR2序列、SEQ ID NO:5的HC CDR3序列、SEQ ID NO:6的轻链(LC)CDR1序列、SEQ ID NO:7的LC CDR2序列和SEQ ID NO:8的LC CDR3序列。

[0262] 实施方案33b是根据实施方案33a所述的组合或试剂盒或组合物,其中所述抗体包含SEQ ID NO:9的HC序列和SEQ ID NO:10的LC序列。

[0263] 实施方案33c是根据实施方案33a或33b所述的组合或试剂盒或组合物,其中所述抗体或其抗原结合片段共价连接到叠氮基团。

[0264] 实施方案33d是根据实施方案33c所述的组合或试剂盒或组合物,其中所述抗体或其抗原结合片段随机地共价连接到所述叠氮基团。

[0265] 实施方案33e是根据实施方案33c所述的组合或试剂盒或组合物,其中所述抗体或其抗原结合片段特异性地共价连接到叠氮基团位点。

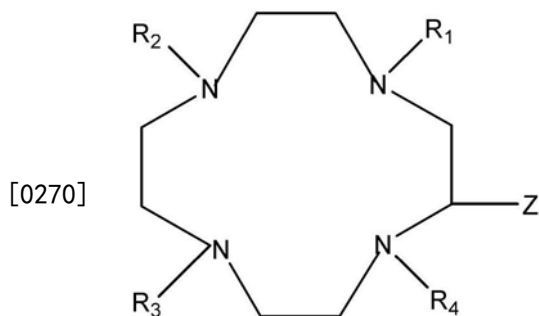
[0266] 实施方案33f是根据实施方案33e所述的组合或试剂盒或组合物,其中所述抗体或其抗原结合片段经由这样的方法共价连接到所述叠氮基团,所述方法包括:用对所述抗体的一个或多个Fc糖基化位点中的一个或多个核心GlcNAc残基之间的 β -1,4键联具有特异性的细菌内切糖苷酶修剪所述抗体或其抗原结合片段,以获得修剪的抗体或其抗原结合片段,以及在糖转移酶,优选地GalT半乳糖基转移酶的存在下使所述修剪的抗体或其抗原结合片段与叠氮糖,优选地UDP-GalNAz叠氮基糖底物反应。

[0267] 实施方案33g是根据实施方案33e所述的组合或试剂盒或组合物,其中所述修饰的抗体或其抗原结合片段通过这样的方法来获得,所述方法包括:用酰胺酶使所述抗体或其抗原结合片段去糖基化,以获得去糖基化的抗体或其抗原结合片段,以及在微生物转谷氨酰胺酶的存在下使所述去糖基化的抗体或其抗原结合片段与叠氮基胺,优选地3-叠氮基丙胺反应。

[0268] 实施方案34是根据实施方案33至33g中任一项所述的组合、试剂盒或组合物,其中

所述放射性金属离子包括 ^{225}Ac 、 ^{111}In 或 ^{89}Zr 。

[0269] 实施方案35是根据实施方案34所述的组合、试剂盒或组合物,其中所述螯合剂包含大环,优选地式(I)的结构:



式(I),

[0271] 其中 R_1 、 R_2 、 R_3 和 R_4 中的每一者独立地为 CHQC_2X ,其中

[0272] Q独立地为氢、 C_1 - C_4 烷基或(C_1 - C_2 烷基)苯基,并且

[0273] X独立地为氢、苄基、 C_1 - C_4 烷基;并且

[0274] Z为 $(\text{CH}_2)_n\text{Y}$,其中

[0275] n为1-10,并且

[0276] Y为共价连接到所述炔烃基团的亲电或亲核部分;

[0277] 另选地,Z为氢;并且

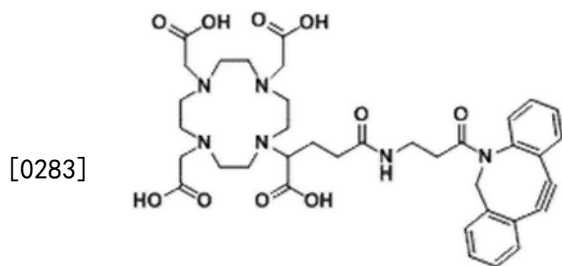
[0278] R_1 、 R_2 、 R_3 和 R_4 中的每一者独立地为 CHQC_2X ,其中

[0279] Q独立地为氢、 C_1 - C_4 烷基或(C_1 - C_2 烷基)苯基,并且

[0280] X独立地为氢、苄基、 C_1 - C_4 烷基或者共价连接到所述炔烃基团的亲电或亲核部分。

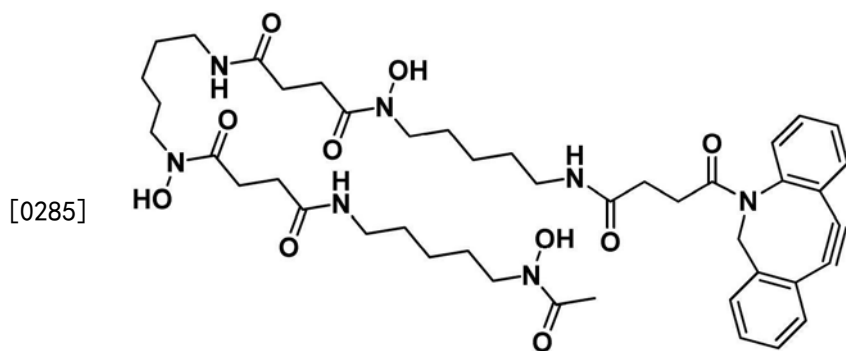
[0281] 实施方案36是根据实施方案35所述的组合、试剂盒或组合物,其中所述亲电或亲核部分经由连接基共价连接到所述炔烃基团。

[0282] 实施方案37是根据实施方案35或36所述的组合、试剂盒或组合物,其中所述螯合部分包含式(II)的结构:



式(II)。

[0284] 实施方案37a是根据实施方案34所述的组合、试剂盒或组合物,其中所述螯合部分包含具有开链配体的螯合剂,优选地所述螯合部分包含式(III)的结构:



式(III)。

[0286] 实施方案38是根据实施方案34至37中任一项所述的组合、试剂盒或组合物,其中所述多肽经由连接基共价连接到叠氮化物。

[0287] 实施方案39是一种在对其有需要的受治疗者中治疗或诊断疾病或障碍,特别是肿瘤性疾病或障碍的方法,包括向所述受治疗者施用根据实施方案25至27d中任一项所述的诊疗剂或根据实施方案28以及33至38中任一项所述的组合。

[0288] 实施方案40是一种在对其有需要的受治疗者中治疗或诊断疾病或障碍,特别是肿瘤性疾病或障碍的方法,包括向所述受治疗者施用根据实施方案31所述的组合物和根据实施方案32所述的组合物,优选地所述多肽为抗体。

[0289] 实施例

[0290] 本发明的以下实施例旨在进一步说明本发明的性质。应当理解,以下实施例不限制本发明,并且本发明的范围由所附权利要求书确定。

[0291] 实施例1:叠氮化物/柄与抗体的随机缀合

[0292] 单克隆抗体(mAb):结合人前列腺特异性膜抗原(PSMA)的人IgG4抗体在本文中称为“抗PSMA mAb”,命名为“PSMB127”,具有SEQ ID NO:3的重链(HC)CDR1序列、SEQ ID NO:4的HC CDR2序列、SEQ ID NO:5的HC CDR3序列、SEQ ID NO:6的轻链(LC)CDR1序列、SEQ ID NO:7的LC CDR2序列和SEQ ID NO:8的LC CDR3序列,并且具有SEQ ID NO:9的HC序列和SEQ ID NO:10的LC序列。使用标准色谱方法表达并纯化抗PSMA mAb。

[0293] 抗PSMA mAb(在本文中称为“对照mAb”)的人IgG4 S228P/F234A/L235A(IgG4-PAA)抗体同种型对照具有SEQ ID NO:1的HC序列和SEQ ID NO:2的LC序列。商业抗体曲妥珠单抗(trastuzumab)(赫赛汀(Herceptin))、西妥昔单抗(cetuximab)(爱必妥(Erbix))、帕妥珠单抗(pertuzumab)(Perjeta)和帕尼单抗(panitumumab)(维克替比(Vectibix))分别购自Roche、Lilly、Roche和Amgen。小鼠抗人Her2 mAb获自BioXCell(目录号BE0277)。曲妥珠单抗、帕妥珠单抗和抗人Her2 mAb与人Her2结合。西妥昔单抗和帕尼单抗与人EGFR结合。

[0294] 缀合:将抗体(1mg/mL-10mg/mL)在10mM乙酸钠pH 5.2、磷酸盐缓冲盐水pH 7或其他相容缓冲液中的储备溶液与20%(v/v)的1M碳酸钠缓冲液pH 9混合至约9的最终pH。将NHS-PEG4-叠氮化物(Thermo目录号26130)溶解于DMSO中至最终浓度为100mM,并且添加0.2%(v/v)的储备液以产生相对于mAb约3-10的摩尔过量。将反应物在22℃下温育10分钟,之后通过添加1M Tris pH 7.5淬灭至最终浓度为50mM Tris。

[0295] 纯化:使用这样的方法将叠氮化物-mAb缀合物纯化并交换到相容缓冲液(PBS; 20mM HEPES 150mM, NaCl pH 7.5;或10mM乙酸钠pH 5.2),该方法诸如具有7K截留分子量

(Thermo)的Zeba脱盐柱,透析;标准蛋白质A亲和色谱法;或另一种相容的方法。在纯化后,使用具有50K截留分子量的Amicon浓缩器(Millipore)将缀合物浓缩至10mg/mL-20mg/mL。

[0296] LC/MS分析:通过LC/MS分析使用Agilent G6224 MS-TOF仪器测定螯合剂:抗体比率(CAR),该仪器配备有Agilent PLRP-S柱(300埃,2.1mmx150mm;目录号PL1912-3301)(表1)。对于质量140kDa-170kDa,使用最大熵算法在m/z范围2000-3200内,使质谱解卷积。

[0297] 分析性尺寸排阻色谱法(SEC):进行分析性SEC以确定抗体和抗体缀合物的低聚状态,并确保缀合过程未导致聚集。使用配备有Tosoh TSKgel G3000SWxl(Tosoh Bioscience#08541)7.8mm×30cm柱的Agilent 1200系列HPLC;流动相1X PBS;流速0.8ml/ml;注入体积15μL;蛋白质浓度0.1mg/mL-2mg/mL。

[0298] 表1:缀合效率

抗体	同种型	CAR
抗PSMA mAb	人IgG4	2.4
对照mAb	人IgG4	2.0
曲妥珠单抗	人源化IgG1	2.5
西妥昔单抗	嵌合人/小鼠IgG1	3.6
帕妥珠单抗	人源化IgG1	2.3
帕尼单抗	人IgG2	3.0
抗人Her2	小鼠IgG2a	3.1

[0300] 实施例2:叠氮化物/柄与非抗体多肽的随机缀合

[0301] 非抗体多肽:转铁蛋白(人全铁转铁蛋白)购自R&D Systems(目录号2914-HT)并溶解于水中至10mg/mL。EGF(人表皮生长因子(EGF))购自Sino Biological(目录号10605-HNAE)。

[0302] 缀合:将多肽(1mg/mL-10mg/mL)在10mM乙酸钠pH 5.2、磷酸盐缓冲盐水pH 7或其他相容缓冲液中的储备溶液与20%(v/v)的1M碳酸钠缓冲液pH 9混合至约9的最终pH。将NHS-PEG4-叠氮化物(Thermo目录号26130)溶解于DMSO中至最终浓度为100mM,并且添加0.2%(v/v)的储备液以产生相对于蛋白质约3-10的摩尔过量。将反应物在22℃下温育10分钟,之后用1M Tris pH 7.5淬灭至最终浓度为50mM。缀合效率示于表2中。

[0303] 表2:缀合效率

蛋白质	螯合剂:蛋白质比率
转铁蛋白	3.6
EGF	1.1

[0305] 实施例3:叠氮基糖到抗体聚糖中的位点特异性掺入

[0306] 用对一个或多个Fc糖基化位点中的核心GlcNAc残基之间的β-1,4键联具有特异性的细菌内切糖苷酶GlycINATOR(Genovis)修剪抗体聚糖,从而使最内的GlcNAc完整保留在Fc上,然后可将其用于位点特异性掺入叠氮基糖。更具体地,将填充到柱(Genovis)中的琼脂糖珠上的固定化GlycINATOR在Tris缓冲盐水(TBS) pH 7.4中进行平衡。将1mL的5mg/mL-10mg/mL mAb添加到树脂中,并在室温下在摇臂上温育1小时。通过以100x g旋转1分钟来洗脱mAb。用0.5mL TBS再洗脱柱3次。将含有修剪的mAb的洗脱液合并,并添加所提供的缓冲添加剂(Genovis)以及UDP-GalNAz叠氮基糖底物和GalT半乳糖基转移酶。将反应物在30℃下

温育过夜。使用mAb Select柱(GE)在AKTA Avant仪器上纯化最终叠氮基mAb。通过LC-MS确认叠氮化物修饰,其中测定CAR恰好为2。

[0307] 实施例4:使用微生物转谷氨酰胺酶(MTG)进行的3-叠氮基丙胺的位点特异性掺入

[0308] 将叠氮基基团位点特异性地安装在抗体上的位置Gln295处,基本上如所述(Dennler等人,Transglutaminase-based chemo-enzymatic conjugation approach yields homogeneous antibody-drug conjugates.Bioconj Chem 2014年3月19日;25(3):第569-78页)。用酰胺酶Rapid PNGase F(New England Biolabs)使抗PSMA mAb去糖基化,该酰胺酶在高甘露糖、杂交体和复合寡糖的最内GlcNAc与天冬酰胺残基之间裂解,并且允许所有N-聚糖(包括来自保守(例如,Fc Asn297)糖基化位点和非保守(例如,Fab N-聚糖)糖基化位点两者的N-聚糖)的完全去糖基化和释放。将10mL于乙酸钠缓冲液pH 5.2中的1mg/mL抗体与5μL的PNGase F在37℃下温育过夜,并通过LC-MS确认去糖基化。通过用Amicon装置(50kDa截留分子量)进行4个循环的浓缩和稀释来去除PNGase F。用于缀合3-叠氮基丙胺(3-APA;Click Chemistry Tools),通过添加2%(v/v)的0.5M HEPES pH 7.5将去糖基化的mAb(0.5mg/mL-1mg/mL)调至pH 7-7.5。添加100当量的3-APA以及5%-10%w/v的一种MTG,即Activa TI转谷氨酰胺酶(Ajinomoto)。将反应物在37℃下温育1-4小时,之后使用标准色谱方法在mAbSelect Sure柱上纯化叠氮化物修饰的mAb。通过LC-MS表征缀合物,并且测定CAR为2。

[0309] 实施例5:放射性金属与双功能螯合剂(BFC)的螯合

[0310] ²²⁵Ac-DOTA-GA-DBCO的合成:²²⁵Ac(NO₃)₃购自Oak Ridge National Laboratory。定制合成1,4,7,10-四氮杂环十二烷,1-(戊二酸)-4,7,10-三乙酸-(3-氨基-丙酸)二苯并环辛炔(DOTA-GA-DBCO)。该合成基于Bernhard等人,Chem.Eur.J.2012,18,7834-7841。使DBCO-胺(3-氨基-1-[(5-氮杂-3,4:7,8-二苯并环辛-1-炔)-5-基]-1-丙酮,Sigma)与DOTA-GA酸酐反应,并且通过反相HPLC纯化产物。

[0311] 使用Capintec CRC-55TW剂量校准器完成锕-225的定量,从而将²²⁵Ac(NO₃)₃溶解于0.1N HCl中以制备10mCi/mL溶液。向塑料小瓶中的四甲基乙酸铵(1M溶液,7.5μL,7.5μmol)、DOTA-GA-DBCO(1mg/mL于水中,2.5μL,3.4nmol)和NaOH(0.1N,2.5μL,0.25μmol)的溶液中添加²²⁵Ac(NO₃)₃(10mCi/mL于0.1N HCl中,5μL,50μCi,0.0038nmol)。通过pH试纸观察到混合物的pH为约6.5。将小瓶置于80℃和290rpm的振荡块上30min,并允许小瓶冷却至室温。

[0312] ¹¹¹In-DOTA-GA-DBCO的合成:0.05M HCl中的¹¹¹InCl₃购自GE Healthcare。向塑料小瓶中的四甲基乙酸铵(1M溶液,7.5μL,7.5μmol)、DOTA-GA-DBCO(1mg/mL于水中,2.5μL,3.4nmol)和HCl(0.1N,5μL)的溶液中添加0.05N HCl中的¹¹¹InCl₃(5μL,在Capintec CRC-55TW剂量校准器中测量为104.3μCi,0.0022nmol)。通过pH试纸观察到混合物的pH为约5.5。将小瓶置于60℃和290rpm的振荡块上30min,并允许小瓶冷却至室温。

[0313] ⁸⁹Zr-DF0-DBCO的合成:⁸⁹Zr草酸盐购自3D Imaging。DF0-DBCO购自Macrocyclics(Plano,TX目录号B-773),将其溶解于DMSO中至0.5mg/mL,并在水中稀释至25μg/mL。

[0314] 将2mCi的Zr-89转移到不含金属的微量离心管中,并且添加1M草酸以达到80μL的总体积。以2μL增量添加12μL的2M碳酸钾,并且用吸移管尖端搅拌混合物直至停止鼓泡。然后添加120μL的1M HEPES,之后添加300μL的水。测试溶液的pH,并且如果有必要的话,添加另外2M碳酸钾以将pH升高至6-6.5。添加136μL的DF0-DBCO储备液(3.4μg),并且将反应物在

室温下温育1小时。通过将0.5μL的反应物点样在TLC Green条(Biodex)上并用20%NaCl洗脱来分析螯合物。在洗脱后,用PerkinElmer Cyclone Plus磷光成像仪扫描条,以确保大部分Zr-89保留在基线处,这表明DFO-DBCO螯合。

[0315] ⁸⁹Zr-DOTA-GA-DBCO的合成:向Waters Sep-pak Light QMA强阴离子交换柱(二醇二氧化硅上的丙烯酸/丙烯酰胺共聚物,表面官能团:C(O)NH(CH₂)₃N(CH₃)₃⁺Cl⁻,300Å孔径,37μm-55μm粒度,230μeq/g离子交换容量)中添加MeCN(6mL),之后添加0.9%盐水(10mL),然后添加水(10mL)。将⁸⁹Zr(ox)₂(2μL,290μCi)于1.0M草酸中的溶液添加到预调理柱中。继而用去离子水(20mL)洗涤柱以去除过量的草酸,之后用1.0M HCl(水溶液)(每次100μL)将⁸⁹ZrCl₄从柱上洗脱出来,以得到400μL的总体积,回收得到248μCi(86%),大部分活性物在级分3中。然后将组合的等分试样蒸发至干。

[0316] 将10μL的DOTA-GA-DBCO(1.0mg/mL于无金属水中,10μg,13.6nmol)添加到⁸⁹ZrCl₄(268μCi,50μL)中。将DOTA-GA-DBCO/⁸⁹Zr溶液在150μL的1.0M HEPES中稀释。将混合物的pH调节至pH 7.5(Pandya等人,Zirconium tetraazamacrocyclic complexes display extraordinary stability and provide a new strategy for zirconium-89-based radiopharmaceutical development.Chem Sci.2017年3月1日;8(3):第2309-2314页)。然后将溶液在90℃下温育60min。通过用1%NH₄OH溶液洗脱的SPC25柱(Sigma Aldrich部件号SPC25120-50G),测定⁸⁹Zr-DOTA-GA-DBCO复合物的收率为98%。未螯合的⁸⁹Zr留在柱上,而⁸⁹Zr-DOTA-GA-DBCO复合物被洗脱出来。

[0317] 实施例6:抗PSMA mAb的点击标记的放射性缀合物的合成

[0318] 关于根据本发明的方法的放射性标记抗体的示意图的示意图参见图1和图2。

[0319] 抗PSMA mAb-二苯并-[1,2,3]-三唑并吡啶辛因-GA-DOTA-225Ac(位点特异性,CAR=2)的合成:将PBS或其他相容缓冲液(10mg/mL-20mg/mL)中的随机或位点特异性叠氮化物修饰的抗体(位点特异性,CAR=2或随机,平均CAR介于1和4之间)添加到如所述产生的²²⁵Ac-DOTA-GA-DBCO溶液中。通过pH试纸,混合物的最终pH为约6.5。轻轻搅拌反应溶液并允许其在室温下静置3h,然后使用以15mL NaOAc缓冲液(10mM,pH 6-6.5)或另一种相容缓冲液预调理的PD-10柱(GE Healthcare)纯化。将反应混合物移取到预调理的PD-10柱的贮存器中,并将洗脱液收集在塑料管中。用NaOAc缓冲液(0.2mL×3)洗涤反应小瓶,将洗涤液移取到PD-10柱的贮存器中,并收集洗脱液。将NaOAc缓冲液连续施加到PD-10柱的贮存器中,并且将洗脱液收集在塑料管中,其中每个管收集约1mL的洗脱液,直至收集到总共10mL的洗脱液。

[0320] 使用柠檬酸盐-H₂O-MeOH溶液作为流动相,通过iTLC-SG(Agilent)评估所收集的每种级分的纯度。组合一种或多种纯级分,以得到10mM NaOAc缓冲液中的最终产物。通过HPLC分析产物溶液的化学和放射性化学纯度。使用标准曲线通过UV吸收测定产物溶液中的抗体浓度。继而使用Capintec CRC-55TW剂量校准器定量产物溶液的活性。

[0321] Ac-225螯合的质量控制:使用二乙三胺五乙酸(DTPA)作为纯化产物的质量控制,采用螯合攻击。将10mM Na₅DTPA水溶液添加到含有²²⁵Ac标记的mAb的样品溶液中,直到[DTPA]/[mAb]=500-1,000。将50mM Na₅DTPA水溶液添加到含有²²⁵Ac标记的mAb的溶液中的纯化产物的等份试样中,直到[DTPA]/[mAb]=50,000-100,000。在室温和290rpm下,将两种混合物置于振荡块上30min。将混合物点样在iTLC-SG上,并用柠檬酸盐-H₂O-MeOH溶液作为

流动相显色。在这些条件下,游离的 ^{225}Ac 迁移到溶剂前沿,并且结合的 ^{225}Ac -mAb保持在基线处。

[0322] 抗PSMA mAb-二苯并-[1,2,3]-三唑并吡啶辛因-GA-DOTA- ^{111}In 的合成:将10mM NaOAc中的随机或位点特异性叠氮化物修饰的抗PSMA mAb(位点特异性,CAR=2或随机,平均CAR介于1和4之间)添加到如所述产生的 ^{111}In -DOTA-GA-DBCO溶液中。轻轻搅拌反应溶液,并且允许其在室温下静置2h,之后使其通过PD-10柱。通过使15mL的NaOAc缓冲液(10mM,pH 6-6.5)通过柱对PD-10柱进行预调理,并移除洗涤液。然后将反应混合物移取到预调理的PD-10柱的贮存器中,并将洗脱液收集在塑料管中。用NaOAc缓冲液(0.2mL $\times$ 3)洗涤反应小瓶,将洗涤液移取到PD-10柱的贮存器中,并收集洗脱液。将NaOAc缓冲液连续施加到PD-10柱的贮存器中,并且将洗脱液收集在塑料管中,其中每个管收集约1mL的洗脱液,直至收集到总共10mL的洗脱液。

[0323] 使用10mM EDTA水溶液(pH=5-6)作为流动相,通过iTLC-SG评估所收集的每种级分的纯度。组合一种或多种纯级分,以得到10mM NaOAc缓冲液中的最终产物。通过HPLC分析产物溶液的化学和放射性化学纯度。使用标准曲线通过UV吸收测定产物溶液中的抗体浓度。继而使用Capintec CRC-55TW剂量校准器定量产物溶液的活性。

[0324] In-111螯合的质量控制:使用DTPA作为纯化产物的质量控制,采用螯合攻击:将10mM Na₅DTPA水溶液添加到含有 ^{111}In 标记的mAb的样品溶液中,直到 $[\text{DTPA}]/[\text{mAb}]=1000-10,000$ 。在室温和290rpm下,将混合物置于振荡块上30min。将混合物点样在iTLC-SG上,并用10mM EDTA水溶液(pH=5-6)作为流动相显色。游离的 ^{111}In 或松散结合的 ^{111}In 迁移到溶剂前沿,并且紧密结合的 ^{111}In -mAb保持在基线处。

[0325] 抗PSMA mAb--二苯并-[1,2,3]-三唑并吡啶辛因-DF0-Zr-89的合成

[0326] 将800 μg 随机叠氮化物修饰的抗PSMA mAb(平均CAR介于1和4之间;相同的程序可用位点特异性叠氮基-mAb进行)于10mM HEPES 50mM NaCl pH 7.5或其他相容缓冲液中添加至如所述产生的 ^{89}Zr -DF0-DBCO溶液中,并在37 $^{\circ}\text{C}$ 下温育1.5小时,然后使其通过PD-10柱。通过使15mL的等渗盐水通过柱对PD-10柱进行预调理,并移除洗涤液。然后将反应混合物移取到预调理的PD-10柱的贮存器中,并将洗脱液收集在塑料管中。将盐水连续施加到PD-10柱的贮存器中,并且将洗脱液收集在塑料管中,其中每个管收集0.5mL的洗脱液,直至收集到总共10mL的洗脱液。用剂量校准器测定每种级分和保留在柱上的材料的活性。合并产物峰(通常为级分4-7)以得到最终产物。通过HPLC分析产物溶液的化学和放射性化学纯度。使用标准曲线通过UV吸收测定产物溶液中的抗体浓度。使用剂量校准器定量产物溶液的活性。

[0327] 抗PSMA mAb-DOTA- ^{89}Zr 的合成

[0328] 将在20mM HEPES 50mM NaCl pH 7.5(10.1mg/mL,200 μL ,约13.5nmol)中通过随机叠氮化物缀合修饰的叠氮化物修饰的抗PSMA mAb缀合物(CAR=1-4)添加到如上所述产生的 ^{89}Zr -DOTA-GA-DBCO溶液中。将混合物的最终pH调节至7.0。将反应溶液在37 $^{\circ}\text{C}$ 下温育2h,之后在PD-10柱(GE Healthcare)上用0.9%盐水、HEPES缓冲液或PBS纯化,以得到64%的产物 ^{89}Zr -DOTA-mAb。

[0329] Zr-89螯合的质量控制:对于Zr-DF0-mAb,仅通过HPLC分析纯化产物。在用DTPA或EDTA攻击时,这些缀合物不保留Zr-89。通过添加EDTA至33mM来攻击Zr-DOTA-mAb,并在室温

下温育过夜。在攻击后通过在PD-10柱上运行来分析缀合物,并且发现保留80%的放射性。

[0330] 实施例7:点击标记的放射性缀合物的分析表征

[0331] 测定放射性化学转化率

[0332] 放射性化学转化率(RA转化率%;参见表3至表5)通过iTLC-SG(使用浸渍有硅胶(SG)的无粘结剂玻璃微纤维色谱纸的瞬时薄层色谱法(iTLC))测定。通过将产物峰的无线电信号(放射性起始材料和副产物的不同保留时间)的积分值除以在对存在于基线与溶剂前沿之间的所有无线电信号峰取积分时获得的值来计算RA转化率%。然后将该产物分数表示为转化率百分比。

[0333] 对于掺入了Ac-225的产物,将含有约0.1 μ Ci-1 μ Ci的²²⁵Ac的样品溶液点样在离底部边缘大约2cm的iTLC-SG条的基线上。使用柠檬酸盐-水-甲醇流动相(20mL 0.4M柠檬酸三钠/3mL 2N HCl/2.3mL MeOH)使iTLC-SG条显色,允许其在室温下干燥,并且在分析之前储存最少6h(达到²²⁵Ac和所有子体核素的长期平衡)。使用利用^{99m}Tc设置的Bioscan AR2000放射性TLC成像扫描仪扫描iTLC-SG。

[0334] 对于In-111螯合物,将含有约0.5 μ Ci-2.5 μ Ci的¹¹¹In的样品溶液点样在离底部边缘大约2cm的iTLC-SG条的基线上。使用10mM EDTA钠pH 5-6作为流动相使iTLC-SG条显色,并且然后允许其在室温下干燥。使用利用In-111设置的Bioscan AR2000放射性TLC成像扫描仪扫描干燥的iTLC-SG。

[0335] 对于Zr-89螯合物,通过将产物峰上的活性除以总活性(包括PD-10柱中的活性)来确定RA转化率%,该总活性通过用剂量校准器计数来确定。

[0336] 测定放射性标记的蛋白质的放射性化学纯度

[0337] Ac-225和In-111螯合物的放射性化学纯度(RA纯度%;参见表3至表4)通过SE-HPLC(尺寸排阻HPLC)测定。对于Ac-225,使用Tosoh TSKgel柱(G3000SWx17.8mm $\times$ 30cm, 5 μ m);用DPBS缓冲液(1x,不含钙和镁)洗脱柱;流速:0.7mL/min;20min运行;室温。在HPLC后,将洗脱液收集在预先编号的小瓶中,其中每个小瓶收集0.5min或1min的洗脱液级分。使容纳洗脱液的小瓶在室温下静置>6h,以允许²²⁵Ac与其子体核素达到长期平衡。然后在Capintec CRC-55TW孔计数器中对每个小瓶中的活性进行计数。基于小瓶中的活性重建放射性色谱图。

[0338] 对于In-111,使用Tosoh TSKgel柱(G3000SWx17.8mm $\times$ 30cm, 5 μ m);用DPBS缓冲液(1x,不含钙和镁)洗脱柱;流速:0.7mL/min;20min运行;室温。使用上述HPLC系统和Perkin Elmer无线流量检测器Radiomatic 625TR完成放射性检测,并且配备有0.5mL流动池,使用In-111设置和Ultima FloTM M混合物,流速为1.4mL/min。

[0339] 对于Zr-89(参见表5),使用Tosoh TSKgel柱(G3000SWx17.8mm $\times$ 30cm, 5 μ m)。用柠檬酸缓冲盐水洗脱柱;流速:1mL/min;20min运行;室温。使用上述HPLC系统和耦接到Bioscan Flow Count仪器的Beckman流过检测器来完成放射性检测。

[0340] 表3:锕-225放射性标记蛋白质

[0341]

mAb	步骤 1*	步骤 2**					CAR
	RA 转化率% (iTLC)	RA 转化率% (iTLC)	RA 纯度% (HPLC)	光谱浓度 ($\mu\text{Ci/mL}$)	光谱质量浓度 ($\mu\text{Ci/mg}$)	总 RA (μCi)	
抗 PSMA mAb (随机叠氮化物)	88%	73%	80%	17.2	74.9	17.2	3-4
抗 PSMA mAb (位点特异性聚糖)	88%	77%	83%	7.2	102.8	7.2	2
抗 PSMA mAb (位点特异性 MTG)	93%	70%	90%	31.3	74.2	31.3	2.0
对照 mAb (随机叠氮化物)	92%	68%	97%	28.1	56.1	28.1	2
帕尼单抗 (随机叠氮化物)	87%	65%	95%	24.9	66.6	24.9	3.0
西妥昔单抗 (随机叠氮化物)	91%	67%	98%	33.7	76.4	33.7	3.6
hEGF (随机叠氮化物)	92%	48%	100%	19.63	1091	19.63	1.1
转铁蛋白 (随机叠氮化物)	94%	57%	78%	24.1	83.7	24.1	3.6
曲妥珠单抗 (随机叠氮化物)	94%	60%	97%	9.88	62.9	9.88	2.5
帕妥珠单抗 (随机叠氮化物)	94%	63%	92%	16.75	49.9	16.75	2.3
抗人 HER2 (随机叠氮化物)	85%	78%	94%	21.5	55.0	21.5	3.1

[0342]

[0343] *步骤1是将铜-225螯合到DOTA-GA-DBCO

[0344] **步骤2是使双官能螯合物与蛋白质进行点击反应

[0345] 表4: 铟-111放射性标记蛋白质

[0346]

mAb	步骤 1*	步骤 2**					
	RA 转化率% (iTLC)	RA 转化率% (iTLC)	RA 纯度% (HPLC)	光谱浓度 (μCi/mL)	光谱质量浓度 (μCi/mg)	总 RA (μCi)	CAR
抗 PSMA mAb (随机叠氮化物)	81%	79%	98%	57.6	139.5	72	2.4
转铁蛋白 (随机叠氮化物)	79%	88%	90%	71.4	410.3	71.4	3.6
帕尼单抗 (随机叠氮化物)	46%	79%	99%	82.0	279	82.0	3.0
西妥昔单抗 (随机叠氮化物)	74%	75%	100%	51.3	290	51.3	3.6

[0347] *步骤1是将铟-111螯合到DOTA-GA-DBCO

[0348] **步骤2是使双官能螯合物与蛋白质进行点击反应

[0349] 表5:用DF0-DBCO进行Zr-89放射性标记抗PSMA mAb

[0350]	mAb	RA 转化率%	RA 纯度% (HPLC)	光谱浓度 (μCi/mL)	光谱质量浓度 (μCi/mg)	总 RA (μCi)	CAR
	抗 PSMA mAb (随机叠氮化物)	47%	100%	131	1171	262	2.4

[0351] 实施例8:修饰的mAb与DOTA-GA-DBCO的点击反应

[0352] 将1mg/mL-10mg/mL的随机和位点特异性叠氮基-mAb (抗PSMA mAb、西妥昔单抗、帕尼单抗、曲妥珠单抗、帕妥珠单抗) 与5x至20x过量的未螯合DOTA-GA-DBCO混合,并在室温或37°C下温育1-24小时。用Zeba脱盐离心柱 (Thermo) 对mAb进行脱盐,并且用Amicon离心浓缩器 (Millipore) 浓缩,用缓冲液再稀释,并且再次浓缩以去除任何剩余的DBCO-DOTA。通过LC-MS确认所有游离叠氮化物与DBCO-DOTA的完全点击反应。

[0353] 实施例9:修饰的mAb与DF0-DBCO的点击反应

[0354] 将1mg/mL-10mg/mL的随机和位点特异性抗PSMA mAb叠氮基-mAb与5x至20x过量的未螯合DOTA-GA-DF0混合,并在室温或37°C下温育1-24小时。用Zeba脱盐离心柱 (Thermo) 对mAb进行脱盐,并且用Amicon离心浓缩器 (Millipore) 浓缩,用缓冲液再稀释,并且再次浓缩以去除任何剩余的DBCO-DF0。通过LC-MS确认所有游离叠氮化物与DBCO-DF0的完全点击反应。

[0355] 实施例10:细胞结合 (FACS)

[0356] 将叠氮化物修饰的抗体、DOTA-DBCO-叠氮化物修饰的抗体和DF0-DBCO-叠氮化物修饰的抗体的细胞结合与表1中所述的缀合物的亲本mAb进行比较。用一定浓度范围内的抗体或缀合物处理表达mAb靶的细胞系,并且通过流式细胞术测量结合。评估帕尼单抗和西妥昔单抗缀合物与EGFR+A431细胞的结合。评估赫塞汀和帕妥珠单抗与HER2+SK-BR-3细胞的结合。评估抗PSMA mAb与PSMA+C4-2b细胞的结合。

[0357] 细胞系:人前列腺癌细胞系C4-2B细胞获自Janssen Oncology (Spring House, PA)。人表皮样癌细胞系A431细胞和人乳腺癌细胞系SK-BR-3细胞获自Janssen BioTherapeutics (Spring House, PA), 其细胞原产于ATCC (Manassas, VA)。将EGFR受体阴性MOLM-13人急性髓性白血病悬浮细胞维持在补充有20%加热灭活胎牛血清(Gibco)的RPMI1640+25mM Hepes (Gibco)中。使细胞在具有10%FBS (Gibco, Waltham, MA)的RPMI1640+25mM Hepes (Gibco, Waltham, MA)中生长。

[0358] 流式细胞术:使用无酶细胞解离缓冲液(Gibco, Waltham, Ma)将细胞从烧瓶中分离,并且通过40um过滤器(Falcon)过滤。在96孔u形底板中每孔接种 5×10^4 个细胞。将细胞与在BSA染色缓冲液(BD Bioscience, San Jose, CA)中稀释的缀合抗体或亲本抗体在4℃下温育1小时。将细胞用染色缓冲液洗涤两次。然后将细胞与AlexaFluor647标记的抗人IgG二级抗体(Jackson ImmunoResearch Laboratories)在4℃下在黑暗中温育30min。将二级抗体在含有3%驴血清(Rockland Immunochemicals,)的染色缓冲液中以1:200稀释。在温育的最后10分钟,将SYTOXTMGreen Nucleic Acid Stain(ThermoFisher)以30nM的最终浓度添加到细胞中。将细胞用染色缓冲液洗涤两次,然后重悬于最终体积为25μL/孔的染色缓冲液中,并在iQue Screener流式细胞仪(Intellicyt)上读数。使用ForeCyt软件分析数据。通过排除具有高核酸染色的事件来测定活细胞。测定活细胞的平均荧光强度(MFI)并在GraphPad Prism 7(GraphPad Software)中作图为抗体浓度的对数对比MFI。将非线性回归曲线拟合添加至数据并计算EC₅₀值。

[0359] 对于所有测试的mAb和缀合物,亲本mAb和修饰的mAb示出类似的细胞结合(表6)。

[0360] 表6:结合靶细胞的mAb/缀合物的EC₅₀ (nM)

[0361]

		mAb	mAb-叠氮化物	mAb-DOTA	mAb-DFO	
	Vectibix	1.3	1.3	0.9		
	西妥昔单抗	0.6	0.2	0.4		
	赫塞汀	2.0	0.9	3.1		
	帕妥珠单抗	2.5	3.3	4.4		
	抗 PSMA mAb (随机叠氮化物)	5.5	4.7	5.4	4.6	
	抗 PSMA mAb (聚糖)	0.7	0.5	0.7		
MTG 样品						
		mAb	去糖基化	mAb-叠氮化物	mAb-DOTA	mAb-DFO
	抗 PSMA mAb (MTG)	4.1	3.4	5.0	6.7	4.5

[0362] 实施例11:In-111细胞结合测定

[0363] 使用表4中描述的In-111放射性标记的蛋白质,用辐射测定法测量细胞结合。对C4-2B细胞(PSMA+和转铁蛋白+)测试抗PSMA mAb和转铁蛋白。对A431细胞(EGFR+)和MOLM-13细胞(EGFR-)测试西妥昔单抗和帕尼单抗。

[0364] 用无酶细胞解离缓冲液(Gibco)分离贴壁细胞。对分离的贴壁细胞和收集的悬浮细胞进行计数并用冷染色缓冲液(BD Biosciences)洗涤。将200μL染色缓冲液中不同数量的细胞添加到微量离心管中并置于冰上。将0.5μCi的In-111标记的蛋白质添加到每个管中,并在冰上温育1小时。用冷PBS (Gibco,)洗涤细胞以去除未结合的抗体,并将其重悬于

500 μ L冷PBS中。将样品转移到计数小瓶中,并通过 γ 计数(Hidex Automatic Gamma Counter)来测定细胞相关的放射性。

[0365] 使用利用以已知量的In-111标记的蛋白质建立的线性回归的CPM值,将研究样品的每分钟计数(CPM)转换成In-111的 μ Ci。使用以下计算将 μ Ci结合值转换成结合的摩尔数: $(\mu\text{Ci结合}/\text{比活性})/\text{MW mAb或蛋白质}$ 。每个数据点是一式两份样品的平均值。

[0366] 点击标记的In-111抗-PSMA mAb和In-111转铁蛋白结合到C4-2B细胞,其中细胞相关的放射性的量随着细胞数量的增加而增加(图3A)。点击标记的In-111抗EGFR抗体帕尼单抗和西妥昔单抗结合到A431细胞,其中细胞相关的放射性的量随着细胞数量的增加而增加;用阴性对照MOLM-13细胞未检测到点击标记的抗EGFR抗体的特异性结合(图3B)。

[0367] 实施例12:细胞摄取测定

[0368] 测定In-111点击标记的抗-PSMA mAb在C4-2B细胞中内化的动力学。

[0369] 将细胞以 3×10^6 个细胞/60mm培养皿(Coming)接种并置于37 $^{\circ}\text{C}$ 潮湿的CO₂培养箱中过夜。移除接种培养基并用2mL冷染色缓冲液(BD Biosciences)替代。然后将培养皿置于冰上。将0.5 μ Ci In-111标记的抗体添加到每个培养皿中,并在冰上温育1小时。用冷PBS(Gibco)洗涤细胞以从细胞表面去除未结合的抗体。在各个时间点,如下所述测定细胞的表面膜结合放射性和细胞内放射性。

[0370] 使用酸洗涤剥离程序剥离表面结合的放射性:向细胞中添加1.5mL剥离缓冲液(50mM甘氨酸、150mM NaCl pH 2.7以及添加至25 μ g/mL的胃蛋白酶(Amresco)),并将培养皿在冰上温育15分钟。将剥离缓冲液转移到计数小瓶中。用冷PBS洗涤细胞,并将洗涤液转移到计数小瓶中。通过 γ 计数(Hidex Automatic Gamma Counter)测定放射性。测定的表面膜结合放射性为剥离缓冲液+PBS洗涤液的总和。

[0371] 通过制备细胞裂解物来测定细胞内放射性:在剥离表面结合的放射性并洗涤细胞后,将1.5mL 1M NaOH(Teknova)添加到细胞中,并将培养皿在冰上温育5分钟。将表面剥离的细胞裂解物转移到计数小瓶中。用冷PBS洗涤培养皿,并将洗涤液转移到计数小瓶中。通过 γ 计数(Hidex Automatic Gamma Counter)测定放射性。测定的细胞内放射性为表面剥离的细胞裂解物+PBS洗涤液的总和。

[0372] 对于时间=0的样品,在初始抗体在冰上结合后立即测定细胞的表面膜结合放射性和细胞内放射性。对于10分钟、30分钟、1小时和2小时的样品,在初始抗体结合后将3mL细胞培养基添加到每个培养皿中,并将培养皿置于37 $^{\circ}\text{C}$ 潮湿的CO₂培养箱中。在每个时间点,将培养皿从培养箱中移除并置于冰上。将细胞培养基转移到计数小瓶中,并用冷PBS洗涤细胞。将PBS洗涤液收集到计数小瓶中。如所述测定细胞的表面膜结合放射性和细胞内放射性。在每个时间点,通过在细胞裂解之前将细胞与PBS而不是剥离缓冲液温育来生成未剥离的样品。这些样品用于评估剥离效率并将结果与剥离的样品相比较。

[0373] 使用来自利用已知量的In-111标记的mAb建立的线性回归的CPM值,将研究样品的CPM转换成 μ Ci In-111。使用下式测定In-111mAb在细胞表面膜(剥离的样品)和细胞内(裂解的样品)的定位百分比:定位 $\%$ = $100 \times (\text{样品}\mu\text{Ci}/\text{平均总}\mu\text{Ci})$,其中总 μ Ci是指添加所有收集的样品,包括温育培养基、PBS洗涤液、甘氨酸冲洗液和裂解的细胞。每个数据点是一式两份样品的平均值。

[0374] 表面结合的In-111从细胞表面快速消失并重新分布在细胞内。剥离技术在时间0

时释放约80%的细胞表面相关的放射性；到温育结束时，仅20%通过剥离释放，并且超过60%的放射性在细胞裂解物中(图4)。

[0375] 实施例13:小鼠肿瘤异种移植模型中的功效

[0376] 抗PSMA mAb-DOTA-Ac-225的剂量范围研究:向雄性NSG小鼠(每组N=8)皮下植入 10^6 个LNCaP细胞,并且使肿瘤生长至 100mm^3 – 150mm^3 。以一系列活性(10nCi、25nCi、70nCi、200nCi)向小鼠静脉内注射单剂量的抗PSMA mAb-叠氮化物-DOTA- ^{225}Ac 或同种型对照即对照mAb-叠氮化物-DOTA- ^{225}Ac 。每只小鼠的注射剂量为最多达总共10 μg 具有冷抗体的蛋白质。一周两次测量肿瘤尺寸和体重。当肿瘤尺寸超过1,500 mm^3 时,或体重减轻超过20%时,将动物安乐死。

[0377] 抗PSMA mAb-DOTA-Ac-225在单次给药之后,特别是在较高放射性剂量下,显示出肿瘤生长抑制,并且在所有剂量下优于同种型对照(图5A)。所有剂量的对照mAb放射性缀合物具有与溶媒对照类似的存活曲线(图5B;表7);抗PSMA mAb缀合物示出明显的剂量响应,其中存活率随着放射性剂量增加而增加(图5C;表7)。在209天后终止研究,其中剩余3只小鼠,所有小鼠来自抗PSMA mAb 200nCi组,并且未示出可检测到的肿瘤。

[0378] 表7:中值存活率

[0379]	剂量	抗 PSMA mAb	同种型对照
	10nCi	61 天	46 天
	25nCi	82 天	48 天
	70nCi	92.5 天	50 天
	200nCi	126.5 天	39.5 天
	溶媒	46	

[0380] 本发明的实施方案旨在仅为示例性的,并且本领域技术人员将认识到或能够使用不超过常规实验来确定本发明的特定程序的许多等同形式。所有此类等同形式被认为是在本发明的范围内并且由以下权利要求书涵盖。

[0381] 本文引用的所有参考文献(包括专利申请、专利和出版物)全文并出于所有目的以引用方式并入本文,其程度就如同每个单独的出版物或专利或专利申请被具体地和单独地表示为全文并出于所有目的以引用方式并入。

<110> Janssen Biotech, Inc.
Vadim Dudkin
Shalom Goldberg
Joseph Erhardt
Rhys Salter

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<130> JBI5149WOPCT1

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<150> 62/599, 830

<151> 2017-12-18

<160> 10

<170> PatentIn 版本 3.5

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[0001] $\langle 211 \rangle$ 447

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Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30

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

Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser
50 55 60

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

Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Leu Tyr Gly Phe Thr Tyr Gly Phe Ala Tyr Trp Gly Gln
100 105 110

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

Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala
130 135 140

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

[0002]

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

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

Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys
195 200 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro
210 215 220

Pro Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val
225 230 235 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
245 250 255

	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	Gln	Glu	Asp	Pro	Glu	
						260				265					270		
	Val	Gln	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	
			275						280					285			
	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Phe	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	
		290						295					300				
	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	
	305					310					315					320	
	Cys	Lys	Val	Ser	Asn	Lys	Gly	Leu	Pro	Ser	Ser	Ile	Glu	Lys	Thr	Ile	
					325					330					335		
	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	
			340						345						350		
[0003]	Pro	Ser	Gln	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	
			355					360					365				
	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	
		370						375				380					
	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	
	385					390					395					400	
	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Arg	Leu	Thr	Val	Asp	Lys	Ser	Arg	
					405					410					415		
	Trp	Gln	Glu	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	
				420					425					430			
	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Leu	Gly	Lys		
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20 25 30

Asn Gly Ile Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro
35 40 45

Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Pro Glu Ser Gly Val Pro
50 55 60

[0004]

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

Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Ile
85 90 95

Ile Glu Asp Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
115 120 125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
130 135 140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145 150 155 160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
165 170 175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
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Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
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Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
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<400> 4

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1 5 10 15

Gly

<210> 5
<211> 15
<212> PRT
<213> 人工序列

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<223> 抗 PSMA mAb (PSMB127) HC CDR3

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<223> 抗 PSMA mAb (PSMB127) LC CDR1

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Arg	Ala	Ser	Gln	Ser	Val	Ser	Ser	Tyr	Leu	Ala
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[0006]

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<211> 7

<212> PRT

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<223> 抗 PSMA mAb (PSMB127) LC CDR2

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1				5		

<210> 8

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<212> PRT

<213> 人工序列

<220>

<223> 抗 PSMA mAb (PSMB127) LC CDR3

<400> 8

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1				5				

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 <211> 451
 <212> PRT
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<220>
 <223> 抗 PSMA mAb (PSMB127) HC

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 1 5 10 15

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

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

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

[0007]

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr 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 Asp Ser Tyr Asp Ser Ser Leu Tyr Val Gly Asp Tyr Phe Asp
 100 105 110

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
 115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu
 130 135 140

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

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

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
180 185 190

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

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

Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly
225 230 235 240

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

[0008]

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln
260 265 270

Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val
275 280 285

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr
290 295 300

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305 310 315 320

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile
325 330 335

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
340 345 350

Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser
355 360 365

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
370 375 380

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
385 390 395 400

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val
405 410 415

Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met
420 425 430

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445

[0009]

Leu Gly Lys
450

<210> 10

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<400> 10

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

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30

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

	Tyr	Asp	Ala	Ser	Asn	Arg	Ala	Thr	Gly	Ile	Pro	Ala	Arg	Phe	Ser	Gly	
	50						55				60						
	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Glu	Pro	
	65					70					75				80		
	Glu	Asp	Phe	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	Arg	Ser	Asn	Trp	Pro	Leu	
					85					90					95		
	Thr	Phe	Gly	Gln	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				
[0010]	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																

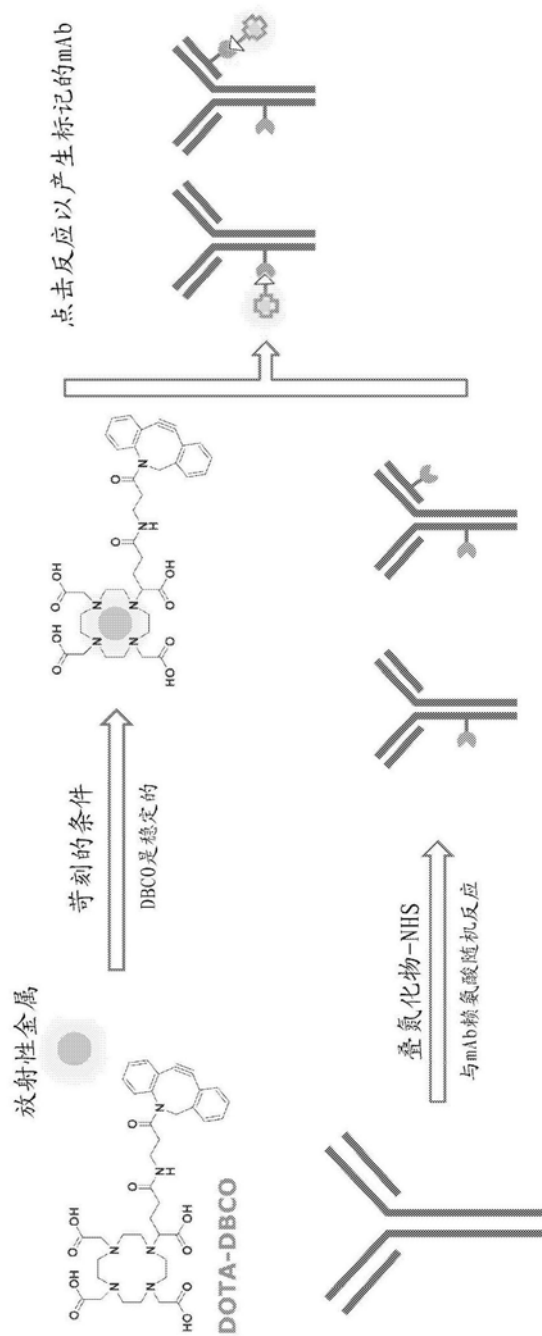


图1

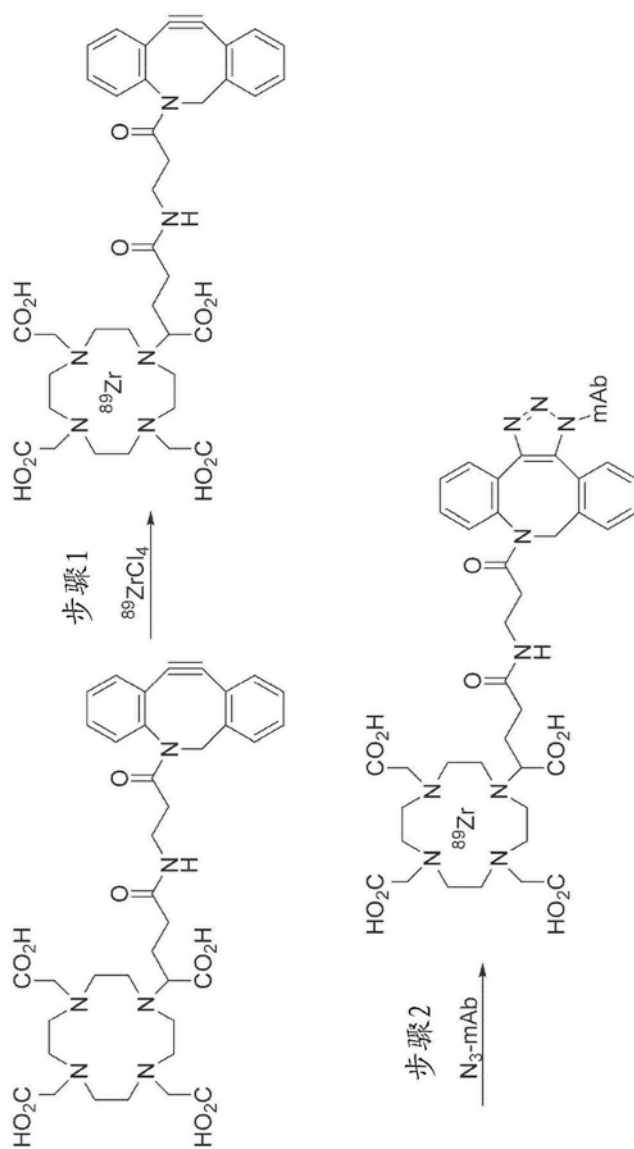


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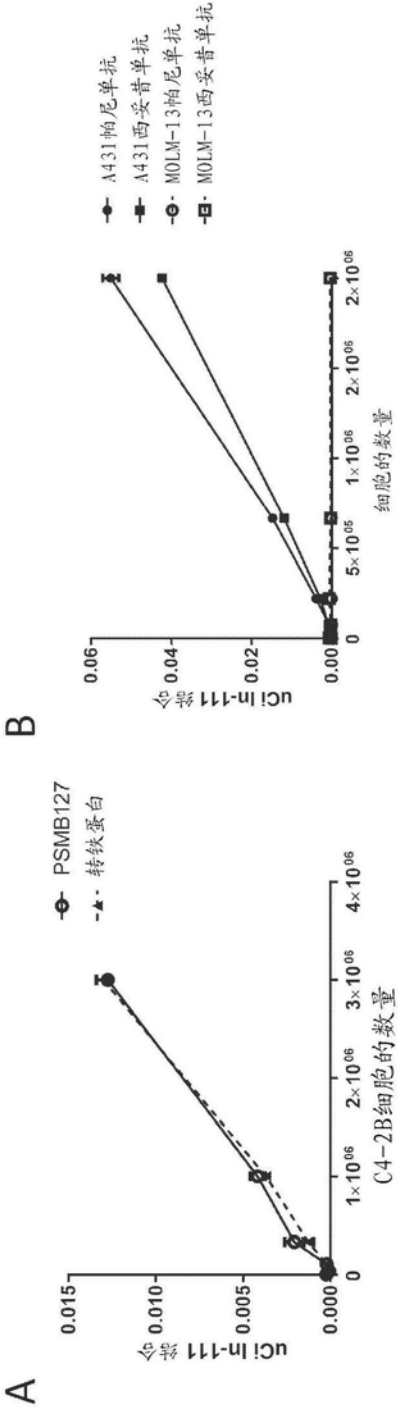


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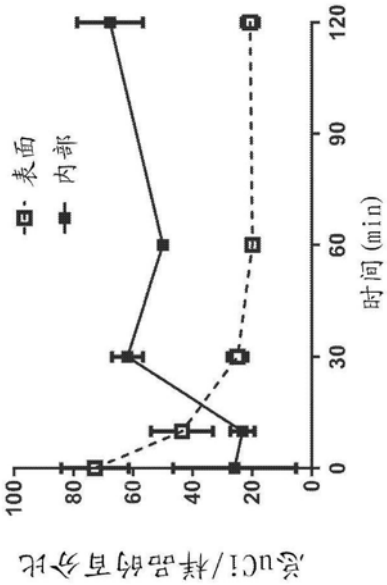


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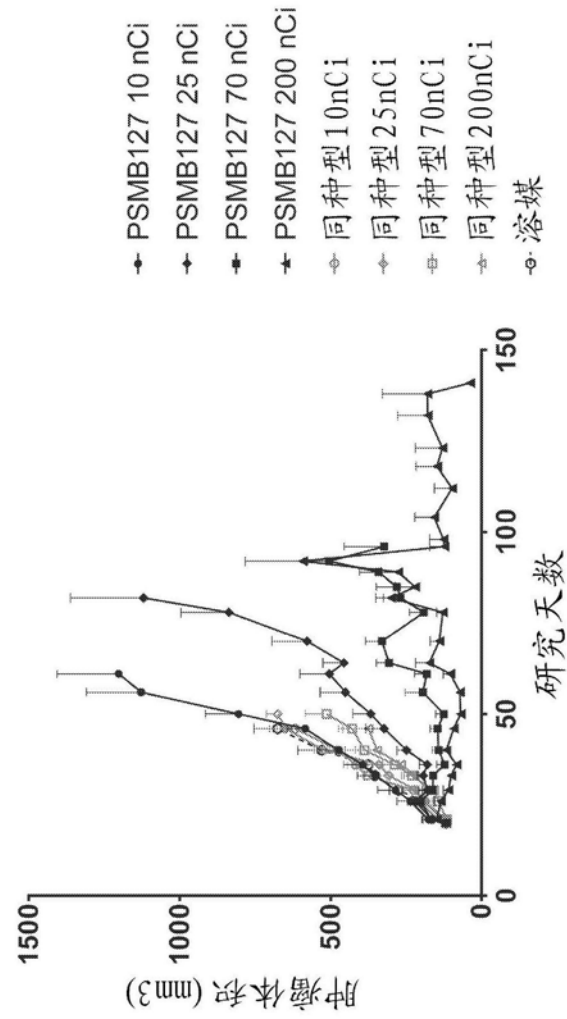


图5A

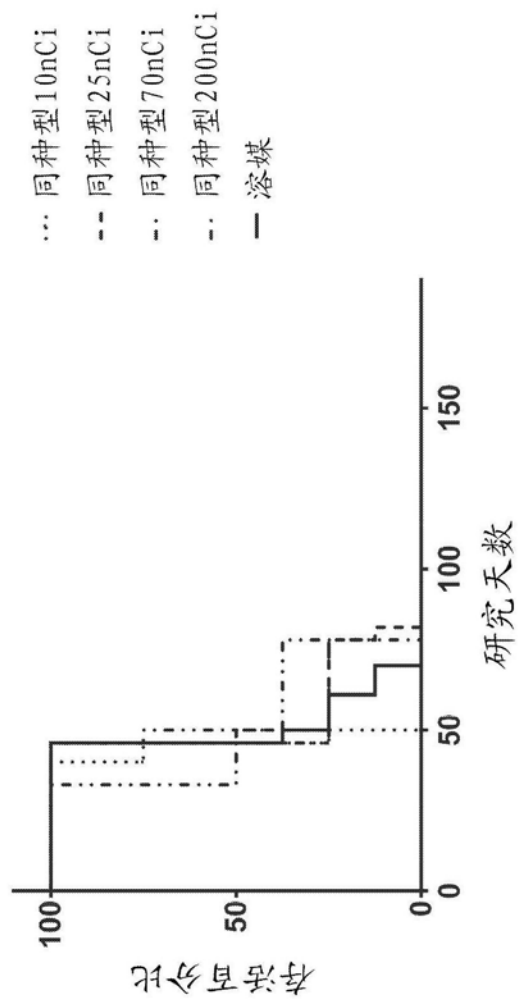


图5B

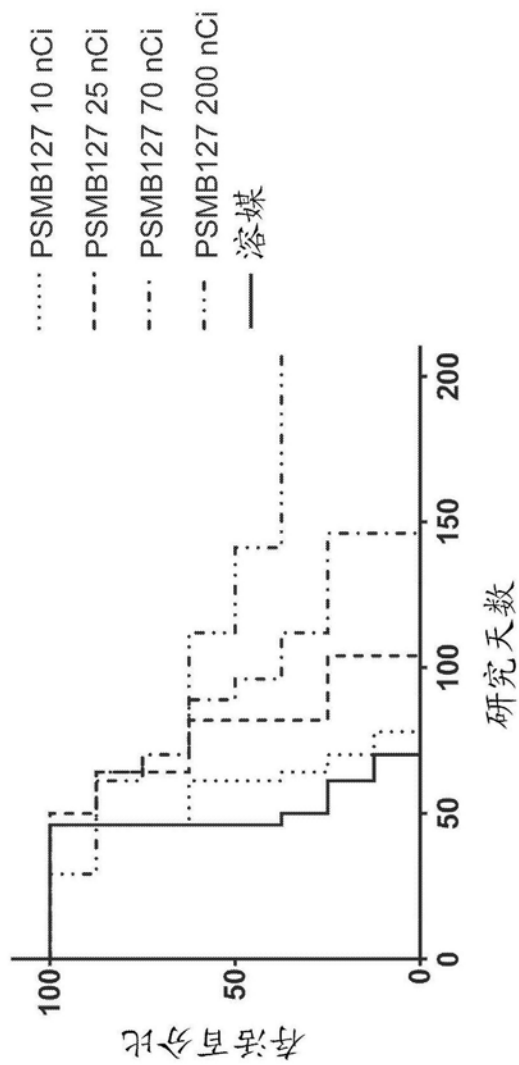


图5C