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TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A SUBMISSION UNDER 35 U.S.C. 371

Attorney Docket No.	U.S. Application No. (if known, see 37 CFR 1.5)
CCPIT-002	

International Application No.	International Filing Date	Priority Date Claimed
PCT/CN2016/073844	February 16, 2016	February 17, 2015

Title of Invention
Antibody-Drug Conjugate

First Named Inventor
Hu, Chaohong

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.

1. ☑ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). NOTE: The express request under 35 U.S.C. 371(f) will not be effective unless the requirements under 35 U.S.C. 371(c)(1), (2), and (4) for payment of the basic national fee, copy of the International Application and English translation thereof (if required), and the oath or declaration of the inventor(s) have been received.
2. ☑ A copy of the International Application (35 U.S.C. 371(c)(2)) is attached hereto (not required if the International Application was previously communicated by the International Bureau or was filed in the United States Receiving Office (RO/US)).
3. An English language translation of the International Application (35 U.S.C. 371(c)(2))
   a. ☑ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
4. An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4))
   a. ☐ is attached.
   b. ☑ was previously filed in the international phase under PCT Rule 4.17(iv).

Items 5 to 8 below concern amendments made in the international phase.

PCT Article 19 and 34 amendments

5. ☐ Amendments to the claims under PCT Article 19 are attached (not required if communicated by the International Bureau) (35 U.S.C. 371(c)(3)).
6. ☐ English translation of the PCT Article 19 amendment is attached (35 U.S.C. 371(c)(3)).
7. ☐ English translation of annexes (Article 19 and/or 34 amendments only) of the International Preliminary Examination Report is attached (35 U.S.C. 371(c)(5)).

Cancellation of amendments made in the international phase

8a. ☐ Do not enter the amendment made in the international phase under PCT Article 19.

8b. ☐ Do not enter the amendment made in the international phase under PCT Article 34.

NOTE: A proper amendment made in English under Article 19 or 34 will be entered in the U.S. national phase application absent a clear instruction from applicant not to enter the amendment(s).

The following items 9 to 17 concern a document(s) or information included.

9. ☑ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
10. ☑ A preliminary amendment.
11. ☑ An Application Data Sheet under 37 CFR 1.76.
12. ☐ A substitute specification. NOTE: A substitute specification cannot include claims. See 37 CFR 1.125(b).
13. ☐ A power of attorney and/or change of address letter.
14. ☑ A computer-readable form of the sequence listing in accordance with PCT Rule 13*ter*.3 and 37 CFR 1.821-1.825.
15. ☐ Assignment papers *(cover sheet and document(s))*. Name of Assignee: _____
16. ☐ 37 CFR 3.73(c) Statement *(when there is an Assignee)*.

This collection of information is required by 37 CFR 1.414 and 1.491-1.492. The information is required to obtain or retain a benefit by the public, which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 15 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop PCT, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

U.S. APPLN. No. (if known – see 37 CFR 1.5)	INTERNATIONAL APPLICATION No.	ATTORNEY DOCKET No.
	PCT/CN2016/073844	CCPIT-002

17. ☐ Other items or information:

The following fees have been submitted.	CALCULATIONS	PTO USE ONLY
18. ☑ Basic national fee (37 CFR 1.492(a)) **$280**	$ 280	
19. ☑ Examination fee (37 CFR 1.492(c)) If the written opinion prepared by ISA/US or the international preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33(1)-(4) ..... **$0** All other situations **$720**	$ 720	
20. ☑ Search fee (37 CFR 1.492(b)) If the written opinion prepared by ISA/US or the international preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33(1)-(4) ..... **$0** Search fee (37 CFR 1.445(a)(2)) has been paid on the international application to the USPTO as an International Searching Authority ..... **$120** International Search Report prepared by an ISA other than the US and provided to the Office or previously communicated to the US by the IB ..... **$480** All other situations **$600**	$ 480	
TOTAL OF 18, 19, and 20 =	$	
☐ Additional fee for specification and drawings filed in paper over 100 sheets (excluding sequence listing in compliance with 37 CFR 1.821(c) or (e) in an electronic medium or computer program listing in an electronic medium) (37 CFR 1.492(j)). Fee for each additional 50 sheets of paper or fraction thereof **$400**		

Total Sheets	Extra Sheets	Number of each addition 50 or fraction thereof (round **up** to a whole number)	RATE	
- 100 =	/ 50 =		x **$400**	$

Surcharge of **$140.00** for furnishing any of the search fee, examination fee, or the oath or declaration after the date of commencement of the national stage (37 CFR 1.492(h)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	23 - 20 =	3	x **$80**	$ 240	
Independent claims	- 3 =		x **$420**	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			**+ $780**	$	
Processing fee of **$140.00** for furnishing the English translation later than 30 months from the earliest claimed priority date (37 CFR 1.492(i)). +				$	
TOTAL OF ABOVE CALCULATIONS =				$ 1720	
☑ **Applicant asserts small entity status.** See 37 CFR 1.27. Fees above are reduced by ½.					
☐ **Applicant certifies micro entity status.** See 37 CFR 1.29. Fees above are reduced by ¾. Applicant must attach form PTO/SB/15A or B or equivalent.					
TOTAL NATIONAL FEE =				$ 860	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property. +				$	
TOTAL FEES ENCLOSED =				$ 860	
				Amount to be refunded:	$
				Amount to be charged:	$

a. ☐ A check in the amount of $_____ to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees.

c. ☑ The Director is hereby authorized to charge additional fees which may be required, or credit any overpayment, to Deposit Account No. 80-0815 as follows:

i. ☐ any required fee.

ii. ☑ any required fee except for excess claims fees required under 37 CFR 1.492(d) and (e) and multiple dependent claim fee required under 37 CFR 1.492(f).

d. ☑ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038. The PTO-2038 should only be mailed or faxed to the USPTO. However, when paying the basic national fee, the PTO-2038 may NOT be faxed to the USPTO.

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NOTE: Where an appropriate time limit under 37 CFR 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the International Application to pending status.

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File)Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013, and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.

NOTE 1: By providing this statement under 37 CFR 1.55 or 1.78, **this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA**.

NOTE 2: A U.S. national stage application may not claim priority to the international application of which it is the national phase. The filing date of a U.S. national stage application is the international filing date. See 35 U.S.C. 363.

Correspondence Address

☑ The address associated with Customer Number: 93726 **OR** ☐ Correspondence address below

Name					
Address					
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Country		Telephone			
Email					

Signature	/James S. Keddie/	Date	August 14, 2017
Name (Print/Type)	James S. Keddie	Registration No. (Attorney/Agent)	48920

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronically Filed

STATEMENT OF ENGLISH TRANSLATION ACCURACY UNDER 37 CFR 1.52(d)(1)	Customer No.	93726
	Attorney Docket No.	CCPIT-002
	Confirmation No.	TBD
	Filing Date	HEREWITH
	First Named Inventor	Hu, Chaohong
	Examiner Name	TBD
	Group Art Unit	TBD
Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Title	"Antibody-Drug Conjugate"

Sir:

Pursuant to 37 CFR 1.52(d)(1), Applicant respectfully acknowledges that the English translation as filed herewith is accurate.

Respectfully submitted,

BOZICEVIC, FIELD & FRANCIS, LLP

Date: August 14, 2017

By: /James S. Keddie/
James S. Keddie
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Antibody-drug conjugate

Technical Field

The present invention relates to an antibody-drug conjugate, in particular to an antibody-drug conjugate in which said antibody is an anti-epidermal growth factor receptor antibody. The present invention also relates to a composition comprising the antibody-drug conjugate, and to a pharmaceutical use of the antibody-drug conjugate.

Background Art

Epidermal growth factor receptor (Epidermal Growth Factor Receptor, EGFR, also known as HER1, c-ErbB1) is a cell surface receptor of epidermal growth factor family, is a transmembrane glycoprotein composed of 1186 amino acid residues, and has a molecular weight of 170kD (Jorissen RN, Walker F, Pouliot N, et al. Epidermal growth factor receptor: mechanisms of activation and signaling. Exp Cell Res, 2003; 284:31-53). EGFR belongs to type I tyrosine kinase receptor subfamily ErbB (ErbB 1-4) and has tyrosine kinase activity. EGFR is stably expressed in many epithelial tissues, including the skin and hair follicles. Abnormal expression of epidermal growth factor receptor or activation caused by receptor mutation may lead to carcinogenesis. There are many solid tumors where over expression of epidermal growth factor receptor are found, such as colorectal cancer, head-neck cancer, lung cancer, ovarian cancer, cervical cancer, bladder cancer and esophageal cancer (Olayioye MA, Neve RM, Lane HA, et al. The EerbB Signaling network: receptor heterodimerzation in development and cancer. The EMBO J, 2000; 19:3159-3167). Growth factors such as transforming growth factor α and epidermal growth factor are endogenous ligands for EGFR. These ligands bind to epidermal growth factor receptor and activate intracellular tyrosine protein kinase activity, initiate a lot of downstream signal transduction pathways, thereby regulating growth and differentiation of normal cells, enhancing invasiveness of tumor cells, promoting angiogenesis and inhibiting apoptosis of tumor cells (Ciardiello F, Tortora G. A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. Clin Cancer Res, 2001; 7:2958-2970). Epidermal growth factor receptor overexpression in tumor and its important roles in the growth and differentiation of tumor cells make epidermal growth factor receptor a promising target for tumor therapy.

At present, there are two anti-epidermal growth factor receptor antibodies in the market,

one is human-mouse chimeric antibody C225 antibody (Erbitux or Cetuximab, ImClone Company (now Eli Lilly Company)), which has a specific binding affinity to epidermal growth factor receptor, can block the binding between a ligand such as EGF or TGFα and a epidermal growth factor receptor, inhibit its phosphorylation and downstream signal transduction, thereby inhibiting tumor cell growth, inducing apoptosis, reducing production of matrix metalloproteinases and vascular endothelial growth factor. The FDA of the United States approved the use of Erbitux for treatment of colorectal cancer in 2004, for treatment of head and neck cancer in 2006, and for other cancer indications in more clinical trials now. Clinically, the overall response rate (ORR) of the combination of Erbitux and irinotecan in treatment of colorectal cancer is 23%, and the ORR of the combination of Erbitux and chemotherapy drug such as fluoropyrimidine in treatment of head and neck cancer is 13%-30%. Because of being a human-mouse chimeric antibody, Erbitux induced an anti-therapeutic antibody response in 3.7% of the patients in the clinical trial.

Another anti-epidermal growth factor receptor antibody is panitumumab (Vectibix, panitumumab, Amgen), which is a fully humanized monoclonal antibody prepared by using transgenic mouse technology, and is free of mouse original protein sequence. The antibody targets epidermal growth factor receptor (EGFR), and was approved by the FDA in September 2006, used in combination with fluoropyrimidine, Oxaliplatin and Irinotecan or for treatment of EGFR positive metastatic colorectal cancer after chemotherapy. In 2006, FDA approved its monotherapy for the treatment of metastatic colorectal cancer (mCRC) with chemotherapy tolerance. However, panitumumab is an IgG2 subtype antibody, and compared with IgG1, IgG2 exhibits significantly decreased biological activities such as CDC activity and ADCC activity; in addition, IgG2 subtype antibodies usually have poor stability. These may be the main reasons that fully humanized antibody panitumumab shows no obvious advantages in clinical effects in comparison with chimeric antibody Erbitux. The overall survival rate (OR) in clinical treatment of colorectal cancer was merely 8% and the progression free survival was only extended by 3.6 months.

At present, large amount of clinical data showed that Erbitux and panitumumab had therapeutic effects only on wild type KRAS (KRAS wild type) with expression of EGFR, but had no tumor growth inhibitory activity to KRAS mutants. Therefore, the Guidelines published by the American Society of Clinical Oncology explicitly point out that anti-EGFR monoclonal antibody drugs are only applicable to KRAS wild type colorectal cancer patients (Allegra CJ, Jessup JM, Somerield MR, Hamilton SR, Hammond EH, Hayes DF, et al. American Society of

Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor monoclonal antibody therapy. J. Clin Oncol. 2009; 27:2091-2096; Bardelli A, Siena S. Molecular mechanisms of resistance to cetuximab and panitumumab in Colorectal, cancer., J, Clin, Oncol. 2010; 28:1254-1261).

Therefore, it is in need in the art to have humanized anti-epidermal growth factor receptor antibody drugs with biological activity, especially antibody drugs, such as antibody-drug conjugates, with curative effects to KRAS mutants, so as to further improve therapeutic efficacy and reduce side effects.

In recent years, the rapid development of antibody-drug conjugates (ADC) has become one of the most advanced biopharmaceutical technologies in recent years due to the lead of monoclonal antibody cancer drugs.

An antibody drug conjugate usually consists of three parts:

1. monoclonal antibody with specific binding to a target;

2. small molecule chemical drug with cytotoxicity;

3. linker that links the small molecule drug and the monoclonal antibody.

An antibody-drug conjugate kills tumor cells by utilizing the target specificity of the monoclonal antibody and the cytotoxicity of the chemical drug. Its mechanism of action is: (1) the antibody-drug conjugate specifically binds to a target antigen expressed on a tumor cell surface by the monoclonal antibody; (2) the complex of the antibody drug conjugate and the target antigen enters the cell via endocytosis mediated by the target antigen; (3) the antibody-drug conjugate degrades in the cell and releases the cytotoxic chemical drug; (4) the cytotoxic chemical drug kills the tumor cell.

Although the mechanism of antibody drug conjugate appears straightforward, it is highly complex and unpredictable whether an antibody drug conjugate becomes a safe and effective drug depending on a variety of factors, such as:

1) characteristics of target: whether a target antigen can be internalized or not, the expression level of the target antigen, differentials of expression level of target antigen between cancer cells and normal cells, and whether the target antigen has a extracellular domain (ECD) that is soluble in blood;

2) characteristics of monoclonal antibody: specificity of the monoclonal antibody to the target antigen (preferably no cross-reaction with other proteins), stability of the monoclonal antibody, and whether the complex of the monoclonal antibody and the target can be

endocytosed into the cell;

3) characteristics of small molecule drug: potency of the cytotoxicity of the small molecule drug, stability thereof in blood, and toxicity of the *in vivo* metabolites of the ADC containing the small molecule drug;

4) characteristics of linker: whether the linker is cleavable or non-cleavable, and stability of the linker in blood;

5) ADC characteristics: whether the complex of ADC and target antigen can be internalized or not, stability of ADC in blood, linker used and number of chemical drugs conjugated to the ADC, the balance between cancer killing activity of ADC and its toxicity.

Therefore, the development of ADC drugs requires substantial amount of experimental exploration and confirmation in the preclinical and clinical studies. Its safety and efficacy are hardly predictable.

Contents of the Invention

The inventors of the present invention have prepared an anti-epidermal growth factor receptor antibody-drug conjugate through a large number of experiments and creative work, and confirmed that it has good biological activities, thereby completing the present invention.

A first aspect of the invention relates to an antibody-drug conjugate, a pharmaceutically acceptable salt thereof, a solvate thereof or a solvate of the salt, comprising an anti-epidermal growth factor receptor antibody covalently linked to a cytotoxic agent.

In one embodiment of the invention, the anti-epidermal growth factor receptor antibody comprises a heavy chain and a light chain, wherein the heavy chain has a variable region comprising CDR1, CDR2 and CDR3 having sequences as shown in SEQ ID NOs: 5 to 7 or mutants thereof, and the light chain has a variable region comprising CDR1, CDR2 and CDR3 having sequences as shown in SEQ ID NOs: 12 to 14 or mutants thereof.

In one embodiment of the invention, the CDR1, CDR2 and CDR3 of the variable region of the light chain of said anti-epidermal growth factor receptor antibody respectively comprise sequences as shown in SEQ ID NOs: 12 to 14, or comprise sequences with identity of greater than 70%, such as greater than 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% 98%, or 99% to sequences as shown in SEQ ID NOs: 12 to 14, for example, sequences having 3, 2 or 1 mutations, deletions or addition of amino acids.

In one embodiment of the invention, the CDR1, CDR2 and CDR3 of the variable region of the heavy chain of said anti-epidermal growth factor receptor antibody respectively comprise sequences as shown in SEQ ID NOs: 5 to 7, or comprise sequences with identity of greater than 70%, such as greater than 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% 98%, or 99% to the sequences as shown in SEQ ID NOs: 5 to 7, for example, sequences having 3, 2 or 1 mutations, deletions or addition of amino acids.

In one embodiment of the invention, FR1, FR2, FR3, FR4 of the variable region of the heavy chain of said anti-epidermal growth factor receptor antibody respectively comprise sequences as shown in SEQ ID NOs: 8 to 11 or mutants thereof.

In one embodiment of the invention, FR1, FR2, FR3, FR4 of the variable region of the heavy chain of said anti-epidermal growth factor receptor antibody respectively comprise sequences as shown in SEQ ID NOs: 8 to 11, or comprise sequences with identity of greater than 70%, for example, greater than 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% to the above sequences.

In one embodiment of the invention, FR1, FR2, FR3, FR4 of the variable region of the light chain of said anti-epidermal growth factor receptor antibody respectively comprise sequences as shown in SEQ ID NOs: 15 to 18, or mutants thereof.

In one embodiment of the invention, FR1, FR2, FR3, FR4 of the variable region of the light chain of said anti-epidermal growth factor receptor antibody respectively comprise sequences as shown in SEQ ID NOs: 15 to 18, or comprise sequences with identity of greater than 70%, for example, greater than 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% to the above sequences.

In one embodiment of the invention, the variable region of the heavy chain of the anti-epidermal growth factor receptor antibody has a sequence as shown in SEQ ID NO: 1.

In one embodiment of the invention, the variable region of the light chain of the anti-epidermal growth factor receptor antibody has a sequence as shown in SEQ ID NO: 2.

In one embodiment of the invention, the heavy chain of the anti-epidermal growth factor receptor antibody has a constant region selected from the group consisting of a human IgG constant region, a human IgM constant region, a human IgA constant region, a human IgD constant region and a mutant thereof.

In one embodiment of the invention, the IgG is selected from IgG1, IgG2, IgG3 and IgG4.

In one embodiment of the invention, constant region of the heavy chain of the

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anti-epidermal growth factor receptor antibody comprises an amino acid sequence as shown in SEQ ID NO: 3, or comprises a sequence with identity of greater than 70%, for example, greater than 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% to the sequence as shown in SEQ ID NO: 3.

In one embodiment of the invention, constant region of the light chain of the anti-epidermal growth factor receptor antibody is a human lambda constant region, a human kappa constant region, or a mutant thereof.

In one embodiment of the invention, constant region of the light chain of the anti-epidermal growth factor receptor antibody comprises an amino acid sequence as set forth in SEQ ID NO: 4, or comprises a sequence with identity of greater than 70%, for example, greater than 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the sequence as shown in SEQ ID NO: 4.

In one embodiment of the present invention, the antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of said salt, has a structure represented by Formula I,

$$Ab\text{-}(L\text{-}D)_p$$

Formula □

Wherein:

Ab represents the anti-epidermal growth factor receptor antibody;

L represents a linker;

D represents the cytotoxic agent;

p represents 1-8, for example 2-6, e.g. 3-5.

In one embodiment of the invention, said cytotoxic agent is selected from the group consisting of chemotherapeutic agents, toxins (e.g., bacterial, fungal, plant, or animal-derived enzymatically active toxins or fragments thereof), radioisotopes, cytokines, antibiotics, enzymes, nanoparticles and biologically active peptides.

In one embodiment of the invention, said cytotoxic agent is selected from the group consisting of Monomethyl auristatin E (MMAE), Monomethyl auristatin F (MMAF), maytansinoid alkaloids (e.g., Maytansine DM1, Maytansine DM4), Calicheamicin, duocarmycin MGBA, doxorubicin, ricin, diphtheria toxin and other toxins, I131, interleukins, tumor necrosis factors, chemokines and nanoparticles.

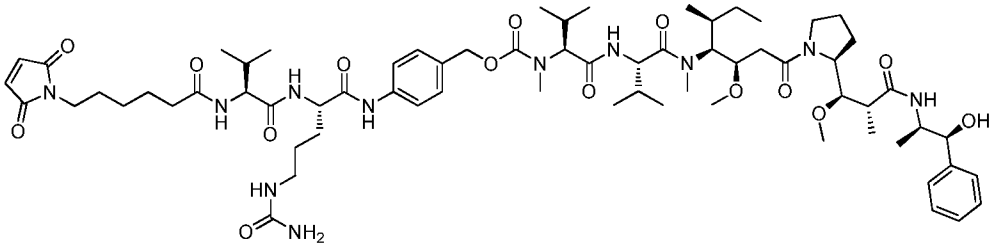
In one embodiment of the invention, said cytotoxic agent is MMAE.

In one embodiment of the invention, said linker is cleavable or non-cleavable.

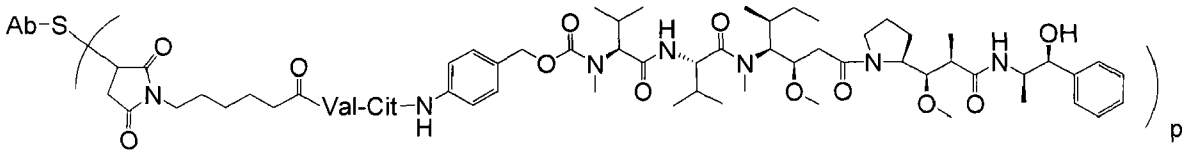
In one embodiment of the present invention, the linker is selected from the group consisting of 6-maleimidocaproyl (MC), maleimidopropionyl (MP), valine-citrulline (val-cit), alanine-phenylalanine (ala-phe), p-aminobenzyloxycarbonyl (PAB), N-succinimidyl 4-(2-pyridylthio)valerate (SPP), N-succinimidyl 4-(N-maleimidomethyl) -cyclohexane-1-carboxylate (SMCC), N-succinimidyl (4-iodo-acetyl) aminobenzoate (SIAB), and 6-maleimidocaproyl-valine-citrulline-p-aminobenzyloxycarbonyl (MC-vc-PAB).

In one embodiment of the present invention, said linker is 6-maleimidocaproyl-valine-citrulline-p-aminobenzyloxycarbonyl (MC-vc-PAB).

In one embodiment of the present invention, the L-D in formula I is vc-MMAE, and the structure is as follows:



In a particular embodiment of the invention, the antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of said salt is represented by the following formula:



Wherein, Ab represents the anti-epidermal growth factor receptor antibody, p is 1-8, e.g. 2-6, e.g. 3-5.

The second aspect of the present invention relates to a composition (e.g., a pharmaceutical composition) comprising the antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to any one of items of the first aspect of the present invention, optionally, further comprising at least one pharmaceutically acceptable carrier, diluent or excipient.

In one embodiment of the present invention, the composition further comprises a known chemotherapeutic agent for the treatment of a tumor, such as Adriamycin, cyclophosphamide and taxane [Taxol and Taxotere], Xeloda, Gemzar, Navelbine, Tamoxifen, aromatase inhibitors (Ruining, Fulong, Arnoldin), 5-FU plus folinic acid,

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camptosar, oxaliplatin, cisplatin, carboplatin, estramustine, Novantrone, prednisone, vincristine (Oncovin), etc., or a combination thereof.

The present invention also relates to a use of the antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to the first aspect of the invention in manufacture of a medicament for prophylaxis and/or treatment of a disease associated with epidermal growth factor receptor (EGFR).

In one embodiment of the invention, the disease associated with epidermal growth factor receptor (EGFR) is a tumor associated with EGFR, such as a tumor associated with overexpression of EGFR, e.g., selected from colon cancer, rectal cancer, head and neck cancer, lung cancer, ovarian cancer, cervical cancer, bladder cancer, esophageal cancer, breast cancer, kidney cancer, prostate cancer, gastric cancer, pancreatic cancer and brain glioma.

In one embodiment of the invention, the tumor is a tumor with KRAS gene mutation, e.g., selected from colon cancer, rectal cancer, lung cancer or pancreatic cancer with KRAS gene mutation.

In one embodiment of the invention, the tumor is a tumor with BRAF gene mutation, e.g., selected from colon cancer, rectal cancer, and lung cancer with BRAF gene mutation.

The invention also relates to a use of the antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to the first aspect of the present invention in manufacture of a medicament for inhibiting tumor angiogenesis, delaying tumor progression, inhibiting tumor growth, or inhibiting tumor cell proliferation.

In one embodiment of the invention, the tumor is selected from colon cancer, rectal cancer, head and neck cancer, lung cancer, ovarian cancer, cervical cancer, bladder cancer, esophageal cancer, breast cancer, renal cancer, prostate cancer, gastric cancer, pancreatic cancer and brain glioma.

In one embodiment of the invention, the tumor is a tumor with KRAS gene mutation, e.g., selected from colon cancer, rectal cancer, lung cancer or pancreatic cancer with KRAS gene mutation.

In one embodiment of the invention, wherein the tumor is a tumor with BRAF gene

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mutation, e.g., selected from colon cancer, rectal cancer, and lung cancer with BRAF gene mutation.

The invention also relates to a method for prophylaxis and/or treatment of a disease associated with epidermal growth factor receptor (EGFR), wherein the method comprising a step of administering to a subject in need a prophylactically and/or therapeutically effective amount of the antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to the first aspect of the invention.

In one embodiment of the invention, the disease associated with epidermal growth factor receptor (EGFR) is a tumor associated with EGFR, such as a tumor associated with overexpression of EGFR, e.g., selected from colon cancer, rectal cancer, head and neck cancer, lung cancer, ovarian cancer, cervical cancer, bladder cancer, esophageal cancer, breast cancer, renal cancer, prostate cancer, gastric cancer, pancreatic cancer and brain glioma.

In one embodiment of the invention, the tumor is a tumor with KRAS gene mutation, for example, colon cancer, colorectal cancer, lung cancer or pancreatic cancer with KRAS gene mutation.

In one embodiment of the invention, the tumor is a tumor with BRAF gene mutation, for example colon cancer, rectal cancer and lung cancer with BRAF gene mutation.

The invention also relates to a method for inhibiting tumor angiogenesis, delaying tumor progression, inhibiting tumor growth, or inhibiting tumor cell proliferation, wherein the method comprising a step of administering to a subject in need a prophylactically and/or therapeutically effective amount of the antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to the first aspect of the invention.

In one embodiment of the invention, wherein the tumor is selected from colon cancer, rectal cancer, head and neck cancer, lung cancer, ovarian cancer, cervical cancer, bladder cancer, esophageal cancer, breast cancer, renal cancer, prostate cancer, gastric cancer, pancreatic cancer and brain glioma.

In one embodiment of the invention, the tumor is a tumor with KRAS gene mutation, for example, colon cancer, colorectal cancer, lung cancer or pancreatic cancer with KRAS gene mutation.

In one embodiment of the invention, the tumor is a tumor with BRAF gene mutation, for example colon cancer, rectal cancer and lung cancer with BRAF gene mutation.

The invention also relates to the antibody-drug conjugate, the pharmaceutically acceptable

salt thereof, the solvate thereof or the solvate of the salt according to the first aspect of the invention, which is used for prophylaxis and/or treatment of a disease associated with epidermal growth factor receptor (EGFR).

In one embodiment of the invention, the disease associated with epidermal growth factor receptor (EGFR) is a tumor associated with EGFR, such as a tumor associated with overexpression of EGFR, e.g., selected from colon cancer, rectal cancer, head and neck cancer, lung cancer, ovarian cancer, cervical cancer, bladder cancer, esophageal cancer, breast cancer, renal cancer, prostate cancer, gastric cancer, pancreatic cancer and brain glioma.

In one embodiment of the invention, the tumor is a tumor with KRAS gene mutation, for example, colon cancer, colorectal cancer, lung cancer or pancreatic cancer with KRAS gene mutation.

In one embodiment of the invention, the tumor is a tumor with BRAF gene mutation, for example colon cancer, rectal cancer and lung cancer with BRAF gene mutation.

The invention also relates to the antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to the first aspect of the invention, which is used for inhibiting tumor angiogenesis, delaying tumor progression, inhibiting tumor growth, or inhibiting tumor cell proliferation.

In one embodiment of the invention, the tumor is selected from colon cancer, rectal cancer, head and neck cancer, lung cancer, ovarian cancer, cervical cancer, bladder cancer, esophageal cancer, breast cancer, renal cancer, prostate cancer, gastric cancer, pancreatic cancer and brain glioma.

In one embodiment of the invention, the tumor is a tumor with KRAS gene mutation, for example, colon cancer, colorectal cancer, lung cancer or pancreatic cancer with KRAS gene mutation.

In one embodiment of the invention, the tumor is a tumor with BRAF gene mutation, for example colon cancer, rectal cancer and lung cancer with BRAF gene mutation.

The anti-epidermal growth factor receptor antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to the present invention has a good inhibition activity on tumor cell growth *in vivo* and *in vitro*, especially obvious inhibition activity on tumor growth of tumor cells with middle level or low level expression of EGFR, as well as has low cytotoxicity; more specifically, the anti-EGFR

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antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to the present invention also has good curative effects on tumors with KRAS gene mutation or BRAF gene mutation, so that it has good application prospects.

The present invention is further described as follows:

In the present invention, the scientific and technical terms used herein have the same meaning as commonly understood by one of ordinary skill in the art, unless otherwise indicated. Also, the terms and laboratory procedures relevant to chemistry of proteins and nucleic acids, molecular biology, cell and tissue culture, microbiology, immunology are all terms and conventional steps that are widely used in the corresponding fields. Meanwhile, for the purpose of better understanding the present invention, definitions and explanations of related terms are provided below.

In the present invention, the term "antibody" refers to an immunoglobulin molecule that is typically composed of two pairs of identical polypeptide chains (each having a "light" (L) chain and a "heavy" (H) chain). The light chain of antibody can be divided into two types: κ and λ. The heavy chain can be divided into five types: μ, δ, γ, α or ε, and the antibody can be therefore divided according to different types of heavy chain into five types: IgM, IgD, IgG, IgA and IgE. Within the light and heavy chains, the variable and constant regions are linked by a "J" region of about 12 or more amino acids, and the heavy chain further comprises a "D" region of about 3 or more amino acids. Each heavy chain consists of a heavy chain variable region (V_H) and a heavy chain constant region (C_H). The heavy chain constant region consists of three domains (C_H1, C_H2 and C_H3). Each light chain consists of a light chain variable region (V_L) and a light chain constant region (C_L). The light chain constant region consists of one domain C_L. The constant regions of antibody may mediate the binding of immunoglobulin to a host tissue or factor, including various cells (e.g., effector cells) of the immune system and component C1q of the complement system. The V_H and V_L regions can also be subdivided into regions with high variability (called complementarity determining regions (CDRs)) among which more conserved regions known as framework regions (FR) are scattered. Each of V_H and V_L consists of three CDRs and 4 FRs arranged in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4 from the amino terminus to the carboxy terminus. The variable regions (V_H and V_L) of each heavy chain/light chain pair form antibody binding sites, respectively. The distribution of amino acids to regions or domains follows the definitions of Kabat Sequences of Proteins of

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Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk (1987) J. Mol. Biol. 196: 901 -917; definition of Chothia et al. (1989) Nature 342: 878-883. The term "antibody" is not limited by any particular method of producing an antibody. For example, it comprises, in particular, recombinant antibodies, monoclonal antibodies, and polyclonal antibodies. The antibodies may be different types of antibodies, e.g., IgG (e.g., IgG1, IgG2, IgG3 or IgG4 subtype), IgA1, IgA2, IgD, IgE or IgM antibodies.

In the present invention, the IgG heavy chain constant region comprises IgG1, IgG2, IgG3 or IgG4. In an embodiment of the invention, the IgG heavy chain constant region is an IgG1 type.

In the present invention, the κ light chain constant region comprises various allotypes, such as Km1, Km1, 2 or Km3.

About amino acid sequence of antibody

In an embodiment of the invention, the heavy chain variable region amino acid sequence of the anti-epidermal growth factor receptor antibody is SEQ ID NO: 1. In an embodiment of the invention, the light chain variable region amino acid sequence of the anti-epidermal growth factor receptor antibody is SEQ ID NO: 2.

In another aspect, the antibody heavy chain variable region amino acid sequence of the invention is at least 70%, preferably at least 75%, preferably at least 80%, preferably 85% more preferably at least 90%, and most preferably at least 95% identical to the sequence of SEQ ID NO: 1.

In another aspect, the antibody light chain variable region amino acid sequence of the invention is at least 70%, preferably at least 75%, preferably at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% identical to the sequence identity of SEQ ID NO: 2.

In an embodiment of the invention, the amino acid sequences of CDRs of the heavy and light chain variable regions of epidermal growth factor receptor antibody are determined as follows:

The amino acid sequences of CDR1, CDR2 and CDR3 of heavy chain are SEQ ID NO: 5 to 7, respectively; the amino acid sequences of CDR1, CDR2 and CDR3 of light chain are SEQ ID NO: 12 to 14, respectively.

In another aspect, the amino acid sequences contained in the CDRs of heavy chains of the

anti-epidermal growth factor receptor antibody may have one or more mutations or additions or deletions of amino acids in SEQ ID NOs: 5 to 7. Preferably, the amino acids for the mutations, additions or deletions are not more than 3 amino acids. More preferably, the amino acids for the mutations, additions or deletions are not more than 2 amino acids. Most preferably, the amino acids for the mutations, additions or deletions are not more than 1 amino acid.

In another aspect, the amino acid sequences contained in the CDRs of light chains of the anti-epidermal growth factor receptor antibody may have one or more mutations or additions or deletions of amino acids in SEQ ID NOs: 12 to 14. Preferably, the amino acids for the mutations, additions or deletions are not more than 3 amino acids. More preferably, the amino acids for the mutations, additions or deletions are not more than 2 amino acids. Most preferably, the amino acids for the mutations, additions or deletions are not more than 1 amino acid.

In an embodiment of the invention, the amino acid sequences of FRs of the heavy and light chain variable regions of the epidermal growth factor receptor antibody are determined as follows:

The sequences of the heavy chain variable regions FR1, FR2, FR3 and FR4 are SEQ ID NO: 8 to 11, respectively. The sequences of the light chain variable regions FR1, FR2, FR3 and FR4 are SEQ ID NO: 15 to 18, respectively.

On the other hand, the amino acid sequences of heavy or light chain variable regions FRs of the anti-epidermal growth factor receptor antibody may have one or more mutations or additions or deletions of amino acids in SEQ ID NOs: 8 to 11 and SEQ ID Nos: 15 to 18. Preferably, the amino acids for the mutations, additions or deletions are not more than 3 amino acids. More preferably, the amino acids for the mutations, additions or deletions are not more than 2 amino acids. Most preferably, the amino acids for the mutations, additions or deletions are not more than 1 amino acid.

The mutants with the above mutations, additions or deletions in antibody or CDR regions or framework regions still retain the ability of specific binding to EGFR.

In an embodiment of the invention, the anti-epidermal growth factor receptor antibody heavy chain constant region amino acid sequence is SEQ ID NO: 3. In an embodiment of the invention, the anti-epidermal growth factor receptor antibody light chain constant region amino acid sequence is SEQ ID NO: 4.

In another aspect, the amino acid sequence of the antibody heavy chain constant region of the invention is at least 70%, preferably at least 75%, preferably at least 80%, preferably at least 85%, more preferably at least 90%, most preferably at least 95%, such as 96%, 97%, 98%,

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99% identical to SEQ ID NO: 3.

In another aspect, the amino acid sequence of the antibody light chain constant region of the invention is at least 70%, preferably at least 75%, preferably at least 80%, preferably at least 85%, more preferably at least 90%, most preferably at least 95%, such as 96%, 97%, 98%, 99% identical to SEQ ID NO: 4.

The monoclonal antibody variants of the present invention can be obtained by conventional genetic engineering methods. Those skilled in the art are fully aware of the methods of transforming DNA molecules using nucleic acid mutations. In addition, nucleic acid molecules encoding heavy and light chain variants can also be obtained by chemical synthesis.

In the present invention, the algorithms for determining the percentage of sequence identity (homology) and sequence similarity are, for example, BLAST and BLAST 2.0 algorithms, which are separately described in Altschul et al. (1977) Nucl. Acid. Res. 25: 3389- 3402 and Altschul et al. (1990) J. Mol. Biol. 215: 403-410. The BLAST and BLAST 2.0 can be used to determine the identity percentages of the amino acid sequences of the invention by using, for example, parameters as described in the literatures or default parameters. The software that performs BLAST analysis can be obtained through the National Biotechnology Information Center.

In the present invention, those amino acid sequences having at least 70% sequence identity to the amino acid sequence comprises polypeptide sequences substantially identical to the amino acid sequence, for example, when the method described herein (e.g., BLAST analysis using standard parameters) is used, those amino acid sequences with at least 70% sequence identity in comparison with the polypeptide sequence of the present invention, preferably at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater sequence identity to the polypeptide sequences of the present invention.

In the present invention, the toxins used for the antibody-drug conjugate include diphtheria toxin A chain, non-binding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, α-sarcin, Aleutites fordii toxic protein, dianthin toxic protein, Phytolacaamericana toxic proteins (PAPI, PAPII and PAP-S), Momordica charantia inhibitors, curcin, crotin, sapaonaria officinalis inhibitors, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and trichothecenes. See also: e.g., WO 93/21232 published on October 28, 1993.

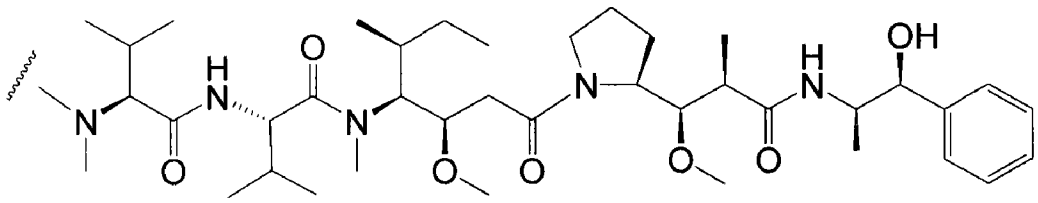
A variety of radionuclides can be used to generate the antibody-drug conjugates. Examples

include ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y, and ^{186}Re.

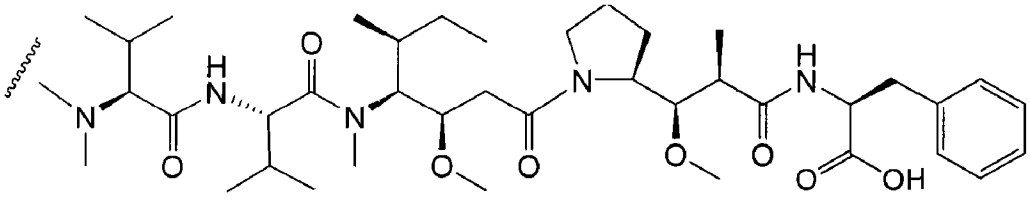
The antibody-cytotoxic agent conjugates can be prepared using a variety of bifunctional protein coupling agents, such as bifunctional derivatives, for example, N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), iminothiolane (IT), imidates (e.g., dimethyl adipimidate dihydrochloride), active esters (e.g., disuccinimidyl suberate), aldehydes (e.g., glutaraldehyde), bisazide compounds (e.g., bis(p-diazidobenzoyl)hexamethylenediamine), bisdiazo compounds (e.g., bis(p-diazobenzoyl)-ethylenediamine), diisothiocyanates (e.g., toluene-2,6-diisocyanate), and double active fluorine compounds (e.g., 1,5-difluoro-2,4-dinitrobenzene). For example, it can be a ricin-containing immunotoxin prepared as described by Vitetta et al. (1987) Science, 238: 1098. The carbon-14 labeled 1-isothiocyanic acid benzyl-3-methyldiethylenetriamine pentaacetic acid (MX-DTPA) is an exemplary chelating agent for coupling radioactive nucleotides with antibodies (WO94/11026). The invention also comprises a conjugate of an antibody and one or more small molecule toxins (e.g., calicheamicin, maytansinoid, dolastatin, auristatin, trichothecene, and CC 1065, as well as toxic derivatives of these toxins).

In some embodiments, the antibody-drug conjugate comprises an anti-epidermal growth factor receptor antibody coupled to dolastatin or a dolastatin peptide analogue and derivative auristatin (U.S. Patent No. 5,635,483; 5,780,588). Dolastatin and auristatin have shown activities of interfering with microtubule kinetics, GTP hydrolysis, and nuclear and cell division (Woyke et al. (2001) Antimicrob. Agents and Chemother. 45 (12): 3580-3584), and have anticancer activity (US 5,663,149) and antifungal activity (Pettit et al. (1998) Antimicrob. Agents Chemother. 42: 2961-2965). The drug modules of dolastatin or auristatin can be attached to an antibody via N (amino) terminus or C (carboxy) terminus of the peptide drug modules (WO 02/088172).

In the present invention, the structure of MMAE is:



In the present invention, the structure of MMAF is:



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In the present invention, an enzyme as cytotoxic agent may be a compound having nucleic acid degradation activity (e.g., ribonuclease or DNA endonuclease, such as deoxyribonuclease; DNA enzyme).

In the present invention, the drug load is represented by p, i.e., the average number of drug modules (i.e., cytotoxic agents) of each antibody in the molecule of formula I: Ab-(L-D)$_p$. The drug load can range from 1 to 20 drug modules (D) per antibody. The ADC of Formula I includes a collection of antibodies conjugated to a range of (1-20) drug modules. The average number of drug modules per antibody from the ADC preparation of coupling reaction can be verified by conventional means, such as mass spectrometry, ELISA assay, and HPLC. The quantitative distribution of ADCs in respect of p can also be determined. In some cases, homogeneous ADCs with p of certain value can be isolated from ADCs with other drug loadings, and purification and validation can be achieved by means such as reverse phase HPLC or electrophoresis.

For some antibody-drug conjugates, p may be limited by the number of attachment sites on the antibody. For example, if the attachment site is a cysteine thiol, the antibody may have only one or several cysteine thiol groups, or may have only one or more thiol groups with sufficient reactivity to attach the linkers. In certain embodiments, a higher drug load, such as $p > 5$, may cause aggregation, insolubility, toxicity, or loss of cell permeability of certain antibody-drug conjugates.

In certain embodiments, the ADCs of the invention have a drug load ranging from 1 to about 8; from about 2 to about 6; from about 3 to about 5; from about 4 to about 5; from about 3.5 to about 4.5; about 4. In fact, it has been shown that some ADCs had an optimal drug load of less than 8, or from about 2 to about 5, for each antibody. See US2005-0238649A1 (which is fully incorporated herein by reference).

In certain embodiments, drug modules less than the theoretical maximum are conjugated to the antibody in the coupling reaction. The antibody may comprise, for example, a lysine residue that does not react with a drug-linker intermediate or a linker reagent. In general, the antibody does not contain a number of free and reactive cysteine thiol groups, which can be linked to a drug moiety; in fact, most of the cysteine thiol groups in the antibody are present in the form of a disulfide bridge. In certain embodiments, the antibody may be reduced with a reducing agent such as dithiothreitol (DTT) or tricarbonyl ethyl phosphine (TCEP) under partial or complete reductive conditions to produce a reactive cysteine thiol group. In certain

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embodiments, the antibody is placed under denaturing conditions to expose a reactive nucleophilic group, such as lysine or cysteine.

The loading (drug/antibody ratio) of ADC may be controlled in different ways, for example, by: (i) limiting the mole number of drug-linker or linker reagent relative to the antibody, (ii) limiting the time or temperature of the coupling reaction, (iii) modifying cysteine thiol moieties or restricting reduction conditions, (iv) performing engineering reconstruction of amino acid sequences of the antibodies by recombinant techniques, such that the number and location of cysteine residues are changed in order to control the number and/or location of the linker-drug attachments. It is to be understood that if more than one nucleophilic group is reacted with a drug-linker intermediate or with a linker reagent and a subsequent drug module reagent, the resulting product is an ADC compound mixture having one or more drug modules attached to the antibody. The average number of drug modules per antibody can be calculated from the mixture by an antibody-specific and drug-specific double-ELISA antibody assay. The various ADC molecules in the mixture can be identified by mass spectrometry and separated by HPLC, for example, hydrophobic interaction chromatography. In certain embodiments, a homogeneous ADC with a single loading value can be isolated from the coupling mixture by electrophoresis or chromatography.

In the present invention, the pharmaceutically acceptable salts of the antibody-drug conjugates include acid addition salts of inorganic acids, carboxylic acids and sulfonic acids, for example, salts of the following acids: hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, toluenesulfonic acid, naphthalene disulfonic acid, acetic acid, trifluoroacetic acid, propionic acid, lactic acid, tartaric acid, malic acid, citric acid, fumaric acid, maleic acid and benzoic acid.

The pharmaceutically acceptable salts of the antibody-drug conjugates of the present invention also include salts of conventional bases, for example (merely exemplified and preferred), alkali metal salts (e.g., sodium salts and potassium salts), alkaline earth metal salts (e.g., calcium salts and magnesium salts) and ammonium salts derived from ammonia or organic amines containing from 1 to 16 carbon atoms, in which the organic amines are, for example (merely exemplified and preferred), ethylamine, diethylamine, triethylamine, ethyl diisopropylamine, monoethanolamine, diethanolamine, triethanolamine, dicyclohexylamine, dimethylaminoethanol, procaine, dibenzamide, N-methylpiperidine, N-methylmorpholine, arginine, lysine and 1,2-ethylenediamine.

In the present invention, the solvate represents these forms of the antibody-drug conjugate of

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the present invention: complexes in solid or liquid form that are formed by coordination of the antibody-drug conjugate with solvent molecules. Hydrate is a specific form of the solvate which has coordinating water molecules. In the present invention, the hydrate is the preferred solvate.

The EGFR-associated tumors that can be preferably treated with the antibody-drug conjugates of the invention include tumors with EGFR overexpression, respiratory tract tumors (e.g., small cell carcinoma and non-small cell carcinoma, bronchial carcinoma), wherein non-small cell lung cancer is particularly preferred; tumors of digestive tracts (e.g., esophagus, stomach, gallbladder, small intestine, large intestine, rectum), wherein intestinal tumor is particularly preferred; tumors of endocrine and exocrine glands (e.g., thyroid and parathyroid, pancreas and salivary glands), wherein pancreatic tumor is particularly preferred; tumors of head and neck regions (e.g., larynx, hypopharynx, nasopharynx, oropharynx, lips, mouth, tongue and esophagus); and/or gliomas.

In the present invention, EGFR overexpression means that the expression level of EGFR is increased as compared with the level of EGFR expression on the surface of normal epithelial cells; specifically, it can be divided into high expression, moderate expression and low expression, for example, DiFi cells are EGFR high expression cell lines, LoVo cells are EGFR moderate expression cell lines, while HT-29 cells are EGFR low expression cell lines (Wild, R., et al., Mol. Cancer Rher 2006: 5 (1), p104-113, Cetuximab preclinical antitumor activity (monotherapy and combination based) is not predicted by relative total or activated epidermal growth factor receptor tumor expression levels).

The antibody-drug conjugates of the present invention may be used in combination with a known chemotherapeutic agent for the treatment of tumors, the chemotherapeutic agent can be, for example, Adriamycin, cyclophosphamide and taxane [Taxol and Taxotere], Xeloda, Gemzar, Navelbine, Tamoxifen, aromatase inhibitors (Arimidex, Femara, Aromasin), 5-FU plus folinic acid, camptosar, oxaliplatin, cisplatin, carboplatin, estramustine, Novantrone, prednisone, Oncovin, etc., or a combination thereof.

In the present invention, "treatment" refers to clinical intervention that attempts to alter the natural course of a treated individual or cell, either for prevention or in the course of clinical pathology. The desired effect of treatment includes the prevention of recurrence or relapse of disease, the alleviation of symptoms, the weakening of any direct or indirect pathological consequences of disease, the prevention of metastasis, the reduction of disease progression rate, the improvement or alleviation of disease status, and the elimination or improvement of prognosis. In some embodiments, the antibody or antibody-drug conjugate of the invention is

used to delay the onset of a disease or condition or to slow down the progression of a disease or condition. The above parameters used to assess the successful treatment and improvement of disease can be easily measured by conventional procedures familiar to physicians. For cancer treatment, efficacy can be measured by, for example, assessing time to progress (TTP) and/or measuring response rate (RR).

In the present invention, "subject" refers to a vertebrate. In certain embodiments, the vertebrate refers to a mammal. The mammal includes, but is not limited to, livestock (such as cattle), pets (such as cats, dogs, and horses), primates, mice and rats. In certain embodiments, the mammal refers to a human.

In the present invention, "effective amount" refers to an amount effective to achieve the desired therapeutic or prophylactic effect at the desired dose and time. The "therapeutically effective amount" of a substance/molecule of the invention may vary depending on factors such as disease state, age, gender and body weight of an individual and the ability of the substance/molecule to elicit a desired response in the individual. The therapeutically effective amount also covers an amount of the substance/molecule of which beneficial effects are superior to any toxic or detrimental effect. "Prophylactically effective amount" refers to an amount effective to achieve the desired prophylactic effect at the desired dose and time. It is generally but not necessary, however, that the prophylactically effective amount will be lower than the therapeutically effective amount since the prophylactic dose is administered to the subject prior to the onset of the disease or early in the disease. In the case of cancer, the therapeutically effective amount of the drug can reduce the number of cancer cells; reduce the tumor volume; inhibit (i.e., slow down to some extent, preferably stop) the cancer cells infiltrating into the surrounding organs; inhibit (i.e., slow down to some extent, preferably stop) tumor metastasis; inhibit to some extent the growth of tumor; and/or alleviate to some extent one or more symptoms associated with cancer.

For the prophylaxis or treatment of the disease, the appropriate dosage of the antibody-drug conjugate of the invention (when used alone or in combination with one or more other therapeutic agents such as chemotherapeutic agents) will depend on the type of disease to be treated, the type of the antibody-drug conjugate, the severity and progression of the disease, the administration of the antibody-drug conjugate that is for the purpose of prevention or treatment, the previous therapy, the patient's clinical history and reactivity with the antibody-drug conjugates, judgment of physicians. Suitably, the antibody-drug conjugate is administered to the patient either once or through a series of treatments. Depending on the type

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and severity of the disease, the initial candidate dose administered to the patient may be about 1 μg/kg to 100 mg/kg (e.g., 0.1 mg/kg to 20 mg/kg) of the antibody-drug conjugate, for example or by one or more separate administrations or by continuous infusion. Depending on the factors described above, the typical daily dose may range from about 1 μg/kg to 100 mg/kg or more. For repeated administrations for several days or more, depending on the conditions, the treatment is usually continued until the desired inhibition of symptoms appears. An exemplary dose of the antibody-drug conjugate may range from about 0.05 mg/kg to about 10 mg/kg. As such, the antibody-drug conjugate of one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, for example, weekly or every three weeks (e.g., such that the patient receives about 2 to about 20 doses, or, for example, about 6 doses of the antibody-drug conjugate). A higher initial loading dose may be administered, followed by one or more doses of lower dose. The process of this therapy is easily monitored by conventional techniques and assays. "Long term" administration refers to the fact that the initial therapeutic effect (activity) is maintained for a longer period of time, in contrast to the short-term pattern, in a continuous mode of administration of the agent. "Intermittent" administration refers to treatment that is not continuous without interruption, but is essentially periodic. Administration in "combination" with one or more other therapeutic agents includes simultaneous (co-) administration and sequential administration in any order.

"Pharmaceutically acceptable carriers" include, when used in the present invention, pharmaceutically acceptable carriers, excipients or stabilizers, which are non-toxic to the cells or mammals to which they are exposed at the dosage and concentration employed. Typically, a physiologically acceptable carrier is a pH buffered aqueous solution. Examples of physiologically acceptable carriers include buffers such as phosphates, citrates and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins such as serum albumin, gelatin or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides and other carbohydrates, including glucose, mannose, sucrose, trehalose or dextrin; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG) and PLURONICS™.

In the present invention, the KRAS gene has the same meaning as the K-RAS gene, which is a member of RAS gene family and encodes K-ras protein, which is related to the generation,

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proliferation, migration, diffusion and angiogenesis of various tumors. Its common mutation sites are codon 12 and codon 13 of exon 2 of K-RAS gene, and codon 61 of exon 3, among which there are 7 mutation hot spots: G12C, G12R, G12S, G12V, G12D, G12A, G13V/D. These seven mutations account for more than 90%. In one embodiment of the invention, the tumor is a tumor with KRAS gene mutation that is associated with EGFR overexpression.

In the present invention, BRAF (v-raf murine sarcoma viral oncogene homolog B1) gene is a proto-oncogene and is a member of the RAF family. Approximately 8% of human tumors have BRAF mutations, and most of the BRAF gene mutations are BRAFV600E mutations that lead to the continued activation of downstream MEK/ERK signaling pathways, which are critical for tumor growth and invasion and metastasis. In one embodiment of the invention, the tumor is a tumor with BRAF gene mutation that is associated with EGFR overexpression.

In the present invention, 20 conventional amino acids and their abbreviations follow their conventional usages. See Immunology-A Synthesis (2nd ed., E. S. Golub and D. R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991)), which is incorporated herein by reference.

Brief Description of the Drawings

Fig.1 shows a HIC-HPLC plot for determining the drug/antibody ratio of antibody-drug conjugates.

Fig.2 shows results of detection of inhibition activity on cells *in vitro* of monoclonal antibody and antibody-drug conjugate, wherein ○ represents BA03 monoclonal antibody, ▲ represents MYK-3 antibody-drug conjugate.

Fig.3 shows inhibition activity of MYK-3 on the growth of colon cancer cell line HT-29, wherein ○ represents BA03 monoclonal antibody and ▲ represents MYK-3 antibody-drug conjugate.

Fig.4 shows inhibition activity of MYK-3 on the growth of glioma cell U87-MG, where ○ represents BA03 monoclonal antibody and ▲ represents MYK-3 antibody drug conjugates.

Fig.5 shows inhibition activity of MYK-3 on the growth of lung cancer cell A549, wherein ○ represents BA03 monoclonal antibody, and ▲ represents MYK-3 antibody-drug conjugate.

Fig.6 shows inhibition activity of MYK-3 on the growth of KRAS mutant colon cancer cell LoVo, in which ◊ represents levofloxacin monoclonal antibody and ▲ represents MYK-3 antibody-drug conjugate.

Fig.7 shows effects of monoclonal antibody and antibody-drug conjugates on volume of HT-29 colon cancer xenografted tumor in mice, in which the data are expressed as the mean ± standard deviation; * indicates P <0.05, ** indicates P <0.01, and *** indicates P <0.001, as compared with the buffer control group.

Fig.8 shows effects of monoclonal antibody and antibody-drug conjugate on body weight of mice of HT-29 colon cancer xenograft model.

Fig.9 shows inhibition activity of MYK-3, monoclonal antibody BA03 and BA03+ equimolar vcMMAE on the growth of colorectal cancer cell DiFi, wherein □ represents monoclonal antibody component BA03 of MYK-3, Δ represents monoclonal antibody BA03 plus vcMMAE in an amount equivalent to MYK-3 drug-loading moles, ◆ represents MYK-3.

Fig.10 shows inhibition activity of MYK-3 on the growth of KRAS mutant colon cancer cell LoVo in nude mice.

Fig.11 shows HIC-HPLC analysis diagrams of BA03-MCC-MMAE, BA03-MC-MMAE and MYK-3.

Fig.12 shows comparison of *in vitro* cell activities of MYK-3 with BA03-MC-MMAE, BA03-MCC-MMAE, wherein □ represents BA03-MC-MMAE, Δ represents BA03-MCC-MMAE, and ◆ represents MYK-3.

Specific Models for Carrying out the Invention

The embodiments of the present invention will be described in details with reference to the following examples, and it will be understood by those skilled in the art that the following examples are intended to be illustrative of the invention and are not to be taken as limiting the scope of the invention. When specific conditions in the examples were not given, they were carried out in accordance with conventional conditions or the conditions recommended by the manufacturers. When reagents or instruments as used were not indicated with the manufacturers, they were conventional products commercially available in the market.

The antibody BA03 of the present invention was BA03 as described in the Chinese invention patent application CN 103772504A, and its preparation method could be seen in Example 3 of this patent application. The sequences of every part of the antibody were as follows:

The sequence of the variable region of heavy chain was:

QVQLQESGPGLVKPSETLSLTCTVSGFSLSNYDVHWVRQAPGKGLEWLGVIWSGG NTDYNTPFTSRLTISVDTSKNQFSLKLSSVTAADTAVYYCARALDYYDYEFAYWGQGT LVTVSS (SEQ ID NO: 1).

wherein the underlined parts were CDR1 (SEQ ID NO: 5), CDR2 (SEQ ID NO: 6), CDR3 (SEQ ID NO: 7), respectively;

the non-underlined parts were FR1 (SEQ ID NO: 8), FR2 (SEQ ID NO: 9), FR3 (SEQ ID NO: 10), FR4 (SEQ ID NO: 11), respectively.

The sequence of the variable region of light chain was:

EIVLTQSPDFQSVTPKEKVTITCRASQSIGTNIHWYQQKPDQSPKLLIKYASESISGIP SRFSGSGSGTDFTLTINSLEAEDAATYYCQQNNEWPTSFGQGTKLEIK (SEQ ID NO: 2).

wherein the underlined parts were CDR1 (SEQ ID NO: 12), CDR2 (SEQ ID NO: 13), CDR3 (SEQ ID NO: 14), respectively;

the non-underlined parts were FR1 (SEQ ID NO: 15), FR2 (SEQ ID NO: 16), FR3 (SEQ ID NO: 17), FR4 (SEQ ID NO: 18), respectively.

The sequence of constant region of heavy chain was:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 3).

The sequence of constant region of light chain was:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES VTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 4).

Example 1: Preparation of antibody-drug conjugate

10 mg of BA03 antibody was buffer exchanged using a 15 mL 30 KD ultrafiltration device into a reduction buffer (25 mM sodium borate, pH 8.0, 25 mM NaCl, 5 mM EDTA) for a total of three times; the final volume was about 1 mL, transferred to a new Eppendorf centrifuge tube (weighed), and weighed; the protein concentration was measured and the total amount of protein was calculated. 2.5 times molar amount of DTT was added to the antibody and incubated at room temperature for 2 hours and continuously mixed. The

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mixture was buffer exchanged using a 15 ml 30 KD ultrafiltration device into a coupling buffer (50 mM Tris, pH 7.2, 150 mM NaCl, 5 mM EDTA) for a total of three times. The concentrated solution was taken and weighed, measured by A280 to determine protein concentration, and the total amount of protein was calculated. 10μl sample was taken and measured by Ellman's method to determine number of free thiol groups.

In addition, the molar concentration of its free thiol groups was calculated by the following formula:

$$C_{thiol} = \frac{A412 \times 113}{b \times 14150} (M)$$

b: optical path length of cuvette (usually 1cm).

The mole number of free thiol groups was calculated from the molar concentration of free thiol groups and the volume of total protein solution.

To the reduced antibody was added 1.1 times the mole number of free thiol groups of vc-MMAE (purchased from Haoyuan Chemical Technology Co., Ltd., No. HY-15575) (dissolved in DMSO), mixed at room temperature and reacted for 2 hours, intermittently mixed. To the reaction system was added with N-acetylcysteine in an amount of 20 times the mole number of vc-MMAE in the reaction solution, mixed, and the mixture was allowed to stand for 5 minutes. The mixture was buffer exchanged using a 15ml 30KD ultrafiltration device into a conjugate stock solution (20 mM sodium citrate (Na-citrate), 0.3% NaCl, 5% Trehalose, 0.05% TWeen-80, pH 6.0) for a total of 3 times. The obtained antibody-drug conjugate MYK-3 sample was stored at 4°C.

Determination of drug/antibody ratio:

The prepared antibody-drug conjugate MYK-3 was analyzed by HIC-HPLC analysis (Jun Ouyang, Drug-To-Antibody (DAR) Ratio and Drug Distribution by Hydrophobic Interaction Chromatography and Reverse Phase High Performance Chromatography, Laurent Ducry (ed.), Antibody Drug Conjugates, Chapter 17, Methods in Molecular Biology, Vol 1045, p275-283) to determine the drug/antibody ratio (DAR), the results were shown in Fig.1, and the average drug load DAR was calculated as 4.1 according to the peak area.

Example 2: Detection of inhibition activity of antibody-drug conjugate MYK-3 on cells *in vitro*

method for detection of inhibition activity on cells:

1.1 After 3-4 times of passages of the thawed cell lines, the culture media were firstly

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discarded, the cells were rinsed with 5mL of DPBS once, and then digested with 3mL of trypsin, resuspended respectively with media, centrifuged with a centrifuge, and the supernatants were discarded. And then the cells were resuspended again with the media, and 0.5 mL samples were taken and counted with a cell counter. The cells were plated on 96-well cell plates (DiFi cells at 10,000 cells/well, HT-29 cells at 5000 cells/well, A549 cells at 2000 cells/well, U87-MG cells at 3000 cells/well, LoVo cells at 4000 cells/well), cultured for 24 hours, then monoclonal antibody BA03 and antibody-drug conjugate MYK-3 as diluted in a series of concentrations were added and incubated in a cell culture incubator for 72 hours. Each well was then added with 20μl of CCK8 color-producing reagent, measured at wave length of 450-650nm with a microplate reader to determine OD450-650 values, and fitted with four parameters logistic model.

Results of the detection of inhibition activity on cells *in vitro*:

The following cell lines were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences.

Regarding the activities in EGFR-overexpressing DiFi cells (human colorectal cancer cells): MYK-3 showed a significantly increased cell growth inhibitory activity than monoclonal antibody BA03, while EC50 decreased by about 10-fold (EC50 of BA03 was 51.9 ng/ml, EC50 of MYK-3 was 5.1 ng/ml), as shown in Figure 2.

Regarding the activities in other tumor cells with moderate expression and low expression of EGFR: MYK-3 showed significant cell growth inhibitory activity relative to monoclonal antibody itself in cancer cells (human colon cancer cells HT29, human lung cancer cell A549, human brain astrocytoma cell line U87-MG) with moderate expression and low expression of EGFR (as shown in Fig.3, Fig.4, Fig.5), wherein EC50 of HT-29 was 611 ng/ml, EC50 of A549 was 28.3 μg/ml, and EC50 of U87-MG was 5.3 μg/ml.

In addition, we also tested activities in KRAS mutant colon cancer cells LoVo (Dunn EF, Ilda M, Myers RA, Hintz KA, Campbell DA, Armstrong EA, Li C and Wheeler DL. Dasatinib sensitizes KRAS mutant colorectal tumors to cetruximab. Oncogene 2011; 30: 561-574) with moderate EGFR expression, and found that MYK-3 showed significant tumor growth inhibitory activity to KRAS mutant colon cancer cell LoVo (as shown in Fig.6, EC50 was 3.2μg/ml), whereas BA03 alone had little inhibitory activity to the cell line.

Example 3: *In vivo* tumor xenograft test in mice

Experimental method of *in vivo* tumor xenograft test in mice:

HT-29 colon cancer cells were cell lines with relatively low expression of EGFR and with BRAF mutations, and the EGFR targeting monoclonal antibody Erbitux as currently marketed for the treatment of colorectal cancer had no growth inhibitory activity to HT-29 cell strains.

HT-29 cell xenograft model: the tumor cells at logarithmic growth phase were collected and counted, then resuspended in 1×PBS. The cell suspension concentration was adjusted to 3×10^7/ml. The tumor cells were inoculated subcutaneously on the right side of back of nude mice with a 1ml syringe (4 gauge needle), 3×10^6/0.1ml/mouse. When the tumor volume reached 150-200 mm^3, the mice were grouped by a randomized block method, 8 mice per group, so as to ensure that the tumor volume and body weight of mice between the groups were uniform. The difference between the mean value of tumor volume in each group and the mean value of tumor volume of all experimental animals was not more than ± 10%. Tail vein administration was performed, once every four days (the 1st, 5th, 9th and 13th day), for a total of 4 times, and the tumor volumes and body weights of mice were regularly measured. There were 8 mice in each administration group.

Experimental results of tumor xenograft study in mice

HT-29 colon cancer xenograft test in mice: there were 5 groups in the test, including buffer solution group as vehicle control (20 mM sodium citrate, 0.3% sodium chloride, 5% trehalose, 0.05% Tween 80, pH 6), BA03 monoclonal antibody group (5 mg/kg), MYK-3 group (1 mg/kg), MYK-3 group (5 mg/kg) and non-binding ADC group (5 mg/kg) (human IgG-vcMMAE conjugate, in which IgG was IgG obtained by purification from human serum, and this conjugate was prepared by the same method as MYK-3). The tumor volume in the mice administrated with MYK-3 was significantly lower than that of the control group, showing a significant anti-tumor growth effect (Fig.7). On the 18th day, for the group of MYK-3 at dose of 5mg/Kg, its tumor growth inhibition rate was up to 54% as compared with the buffer group, and its tumor growth inhibition rate was up to 46% as compared with the group of monoclonal antibody BA03 at the same dose, and its tumor growth inhibition rate was up to 42% as compared with the non-binding ADC.

Body weight of mice: the body weight of mice administrated with MYK-3 showed no significant change as compared with the control group (see Fig.8), indicating that MYK-3 had not toxic effect of reducing body weight of mice.

Example 4: Inhibitory activities of MYK-3, monoclonal antibody BA03 and BA03 + equimolar vcMMAE on growth of colorectal cancer cell DiFi

The inhibitory activities of MYK-3, monoclonal antibody BA03 and BA03 + equimolar vcMMAE on the growth of colorectal cancer cell DiFi were determined according to the method of Example 2, and the experimental results were shown in Fig.9, in which EC50 values were 8.4 ng/mL, 65.8 ng/ML and 68.2 ng/mL, respectively.

It can be seen from the figure that monoclonal antibody BA03 showed a certain inhibitory activity on the growth of colorectal cancer cell DiFi with EGFR overexpression; and there was not significant difference in the activity between the BA03 plus free vcMMAE in an amount equivalent to the loading mole number of MYK-3 and BA03 alone. However, MYK-3 as an ADC molecule that was formed by conjugating BA03 with vcMMAE showed an inhibitory activity on growth of DiFi colorectal cancer cells much higher than those of monoclonal antibody BA03 itself and BA03 plus free vcMMAE in an amount equivalent to the loading mole number of MYK-3, and EC50 values were elevated by about 8 times.

Example 5: Inhibitory activity of MYK-3 on the growth of transplanted tumor of KRAS mutant colon cancer cell LoVo in nude mice

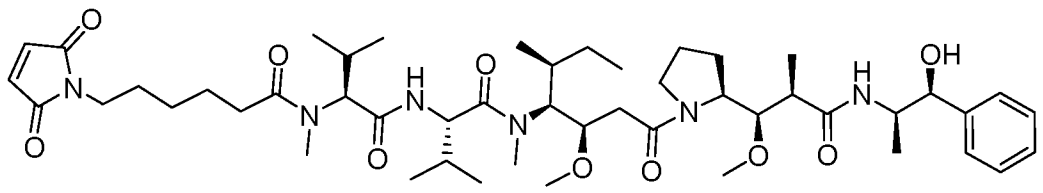
The inhibitory activity of MYK-3 on the growth of transplanted tumor of KRAS mutant colon cancer cell LoVo in nude mice was determined according to the model construction (tumor cells were inoculated at 2×10^6/0.1ml/mouse) and administration methods of Example 3. There were 6 groups in the experiment, including diluted buffer solution group as vehicle control (20 mM sodium citrate, 0.3% sodium chloride, 5% trehalose, 0.05% Tween 80, pH 6), Erbitux monoclonal antibody group (3 mg/kg), MYK-3 groups (three doses: 0.3 mg/kg, 1 mg/kg, 3 mg/kg), and non-binding control ADC group (3 mg/kg), wherein the non-binding control ADC represented non-binding ADC control (which was anti-CD20 mAb-vcMMAE) with the same loading, and this conjugate was prepared by the same method as MYK-3. The experimental results were shown in Fig.10.

It can be seen from the figure that MYK-3 at dose of 3 mg/kg exhibits complete inhibition on the growth of LoVo cell tumors, and MYK-3 at dose of 1 mg/kg shows more potent activity in comparison to the marketed Erbitux at dose of 3 mg/kg.

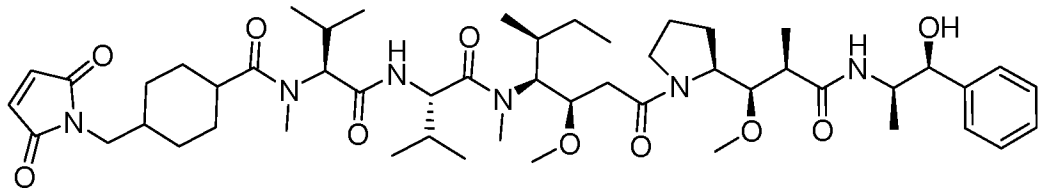
Example 6: Comparison of inhibition activity on cells *in vitro* between MYK-3 and BA03-MC-MMAE, BA03-MCC-MMAE

The structures of MC-MMAE and MCC-MMAE are shown as follows:

MC-MMAE:



MCC-MMAE:



MC-MMAE and MCC-MMAE were prepared by referring to the method of Example 1.

Determination of drug/antibody ratio: HIC-HPLC assay was used for determination of drug/antibody ratio, and specific method could be seen in Example 1. The measured drug/antibody ratios for MYK-3 (i.e., BA03-vcMMAE), BA03-MC-MMAE and BA03-MCC-MMAE were all of 3.9, see also Fig.11.

The method for detecting inhibition activity on cells *in vitro* can refer to Example 2. In short, a number of DiFi cells were taken, inoculated in 96-well plates, cultured for 24 hours, then three samples BA03-MC-MMAE, BA03-MCC-MMAE, MYK-3 in different concentrations of a serial dilution were added. After further incubation for 96 hours, CCK-8 reagent was added for color development, the OD value of each well of the 96-well plates was read by a microplate reader, the number of viable cells in samples of different concentrations was measured, and the inhibitory effect of the samples on the proliferation of DiFi cell lines was determined. The experimental results were shown in Fig.12, in which the EC50 values of BA03-MC-MMAE, BA03-MCC-MMAE and MYK-3 were 72.6 ng/mL, 71.6 ng/mL and 9.8 ng/mL, respectively.

It can be seen from the figure that the cell proliferation inhibitory activity of MYK-3 is significantly higher than those of BA03-MC-MMAE and BA03-MCC-MMAE. In fact, the cell proliferation inhibitory activities of BA03-MC-MMAE and BA03-MCC-MMAE were similar to the activity of monoclonal antibody BA03 itself. This shows that when the same monoclonal antibody and cytotoxic small molecules are used, the use of different linkers may cause significant differences in ADC activity.

While specific embodiments of the present invention have been described in detail, those skilled in the art will understand that various modifications and substitutions can be made to

those details according to all teachings that have been disclosed, and all of these changes fall within the scope of the present invention. The full scope of the invention is given by the appended claims and any equivalents thereof.

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What is claimed is:

1. An antibody-drug conjugate, a pharmaceutically acceptable salt thereof, a solvate thereof or a solvate of the salt, comprising an anti-epidermal growth factor receptor antibody covalently linked to a cytotoxic agent.

2. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to claim 1, wherein the anti-epidermal growth factor receptor antibody comprises a heavy chain and a light chain, wherein the heavy chain has a variable region comprising CDR1, CDR2 and CDR3 having sequences as shown in SEQ ID NOs: 5 to 7 or mutants thereof, and the light chain has a variable region comprising CDR1, CDR2 and CDR3 having sequences as shown in SEQ ID NOs: 12 to 14 or mutants thereof.

3. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to claim 2, wherein FR1, FR2, FR3, FR4 of the variable region of the heavy chain of the anti-epidermal growth factor receptor antibody respectively comprise sequences as shown in SEQ ID NOs: 8 to 11 or mutants thereof.

4. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to claim 2, wherein FR1, FR2, FR3, FR4 of the variable region of the light chain of the anti-epidermal growth factor receptor antibody respectively comprise sequences as shown in SEQ ID NOs: 15 to 18, or mutants thereof.

5. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to any one of claims 1 to 4, wherein the heavy chain of the anti-epidermal growth factor receptor antibody has a constant region selected from the group consisting of a human IgG constant region, a human IgM constant region, a human IgA constant region, a human IgD constant region and a mutant thereof.

6. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to claim 5, wherein the IgG is selected

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from the group consisting of IgG1, IgG2, IgG3 and IgG4.

7. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to claim 5, wherein the constant region of the heavy chain of the anti-epidermal growth factor receptor antibody comprises an amino acid sequence as shown in SEQ ID NO: 3, or comprises a sequence with an identity of greater than 70%, for example, greater than 75%, 80%, 85%, 90%, 95%, or 99% to the sequence as shown in SEQ ID NO: 3.

8. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to any one of claims 1 to 7, wherein the light chain of the anti-epidermal growth factor receptor antibody has a constant region selected from the group consisting of a human lambda constant region, a human kappa constant region and a mutant thereof.

9. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to claim 8, wherein the constant region of the light chain of the anti-epidermal growth factor receptor antibody comprises an amino acid sequence as shown in SEQ ID NO: 4, or comprises a sequence with an identity of greater than 70%, for example, greater than 75%, 80%, 85%, 90%, 95%, or 99% to the sequence as shown in SEQ ID NO: 4.

10. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to any one of claims 1 to 9, which has a structure as shown in Formula I,

$$Ab\text{-}(L\text{-}D)_p$$

Formula □

wherein:

Ab represents the anti-epidermal growth factor receptor antibody;

L represents a linker;

D represents the cytotoxic agent;

p represents 1-9, for example 2-6, e.g. 3-5.

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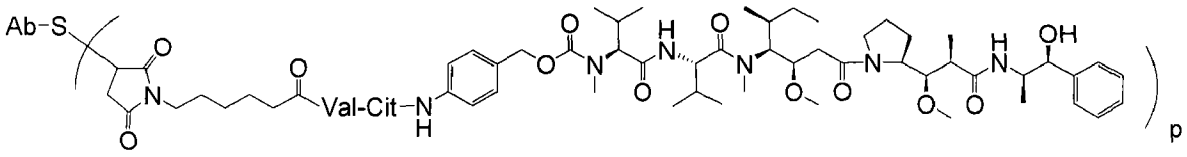
11. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to any one of claims 1 to 10, wherein the cytotoxic agent is selected from the group consisting of chemotherapeutic agents, toxins, radioisotopes, cytokines, antibiotics, enzymes, nanoparticles and biologically active peptides.

12. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to claim 11, wherein the cytotoxic agent is selected from the group consisting of Monomethyl auristatin E (MMAE), Monomethyl auristatin F (MMAF), maytansinoid alkaloids (e.g., Maytansine DM1, Maytansine DM4), Calicheamicin, duocarmycin MGBA, doxorubicin, ricin, diphtheria toxin and other toxins, I131, interleukins, tumor necrosis factors, chemokines and nanoparticles, etc., preferably MMAE.

13. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to claim 10, wherein the linker is cleavable or non-cleavable.

14. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to claim 13, wherein the linker is selected from the group consisting of 6-maleimidocaproyl (MC), maleimidopropionyl (MP), valine-citrulline (val-cit), alanine-phenylalanine (ala-phe), p-aminobenzyloxycarbonyl (PAB), N-succinimidyl 4-(2-pyridylthio)valerate (SPP), N-succinimidyl 4-(N-maleimidomethyl) -cyclohexane-1-carboxylate (SMCC), N-succinimidyl (4-iodo-acetyl) aminobenzoate (SIAB), and 6-maleimidocaproyl-valine-citrulline-p-aminobenzyloxycarbonyl (MC-vc-PAB), preferably 6-maleimidocaproyl-valine-citrulline-p-aminobenzyloxycarbonyl (MC-vc-PAB).

15. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to any one of claims 1 to 14, which is:



wherein, Ab represents the anti-epidermal growth factor receptor antibody, p is 1-8, e.g. 2-6, e.g. 3-5.

16. A composition, which comprises the antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to any one of claims 1 to 15, optionally, further comprises at least one pharmaceutically acceptable carrier, diluent or excipient.

17. A use of the antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to any one of claims 1 to 15 in manufacture of a medicament for prophylaxis and/or treatment of a disease associated with epidermal growth factor receptor (EGFR).

18. The use according to claim 16, wherein the disease associated with epidermal growth factor receptor (EGFR) is a tumor associated with EGFR, such as a tumor associated with overexpression of EGFR, e.g., selected from colon cancer, rectal cancer, head and neck cancer, lung cancer, ovarian cancer, cervical cancer, bladder cancer, esophageal cancer, breast cancer, kidney cancer, prostate cancer, gastric cancer, pancreatic cancer and brain glioma.

19. A use of the antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to any one of claims 1 to 15 in manufacture of a medicament for inhibiting tumor angiogenesis, delaying tumor progression, inhibiting tumor growth, or inhibiting tumor cell proliferation.

20. The use according to claim 19, wherein the tumor is selected from colon cancer, rectal cancer, head and neck cancer, lung cancer, ovarian cancer, cervical cancer, bladder cancer, esophageal cancer, breast cancer, renal cancer, prostate cancer, gastric cancer, pancreatic cancer and brain glioma.

21. The use according to claim 18 or 20, wherein the tumor is a tumor with KRAS gene mutation, e.g., colon cancer, rectal cancer, lung cancer or pancreatic cancer with KRAS gene mutation.

22. The use according to claim 18 or 20, wherein the tumor is a tumor with BRAF gene

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mutation, e.g., colon cancer, rectal cancer, and lung cancer with BRAF gene mutation.

23. A method for prophylaxis and/or treatment of a disease associated with epidermal growth factor receptor (EGFR), wherein the method comprises a step of administering to a subject in need a prophylactically and/or therapeutically effective amount of the antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to any one of claims 1 to 15.

24. The method according to claim 23, wherein the disease associated with epidermal growth factor receptor (EGFR) is a tumor associated with EGFR, such as a tumor associated with overexpression of EGFR, e.g., selected from colon cancer, rectal cancer, head and neck cancer, lung cancer, ovarian cancer, cervical cancer, bladder cancer, esophageal cancer, breast cancer, renal cancer, prostate cancer, gastric cancer, pancreatic cancer and brain glioma.

25. A method for inhibiting tumor angiogenesis, delaying tumor progression, inhibiting tumor growth, or inhibiting tumor cell proliferation, wherein the method comprises a step of administering to a subject in need a prophylactically and/or therapeutically effective amount of the antibody-drug conjugate, the pharmaceutically acceptable salt , the solvate thereof or the solvate of the salt according to any one of claims 1 to 15. thereof

26. The method according to claim 25, wherein the tumor is selected from colon cancer, rectal cancer, head and neck cancer, lung cancer, ovarian cancer, cervical cancer, bladder cancer, esophageal cancer, breast cancer, renal cancer, prostate cancer, gastric cancer, pancreatic cancer and brain glioma.

27. The method according to claim 24 or 26, wherein the tumor is a tumor with KRAS gene mutation, for example, colon cancer, colorectal cancer, lung cancer or pancreatic cancer with KRAS gene mutation.

28. The method according to claim 24 or 26, wherein the tumor is a tumor with BRAF gene mutation, for example colon cancer, rectal cancer and lung cancer with BRAF gene mutation.

29. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to any one of claims 1 to 15, which is used for prophylaxis and/or treatment of a disease associated with epidermal growth factor receptor (EGFR).

30. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to claim 29, wherein the disease associated with epidermal growth factor receptor (EGFR) is a tumor associated with EGFR, such as a tumor associated with overexpression of EGFR, e.g., selected from colon cancer, rectal cancer, head and neck cancer, lung cancer, ovarian cancer, cervical cancer, bladder cancer, esophageal cancer, breast cancer, renal cancer, prostate cancer, gastric cancer, pancreatic cancer and brain glioma.

31. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to any one of claims 1 to 15, which is used for inhibiting tumor angiogenesis, delaying tumor progression, inhibiting tumor growth, or inhibiting tumor cell proliferation.

32. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to claim 31, wherein the tumor is selected from colon cancer, rectal cancer, head and neck cancer, lung cancer, ovarian cancer, cervical cancer, bladder cancer, esophageal cancer, breast cancer, renal cancer, prostate cancer, gastric cancer, pancreatic cancer and brain glioma.

33. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to claim 30 or 32, wherein the tumor is a tumor with KRAS gene mutation, for example, colon cancer, colorectal cancer, lung cancer or pancreatic cancer with KRAS gene mutation.

34. The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to claim 30 or 32, wherein the tumor is a tumor with BRAF gene mutation, for example colon cancer, rectal cancer and lung cancer with BRAF gene mutation.

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Abstract

The present invention relates to an antibody-drug conjugate, in particular, to an antibody-drug conjugate targeting an epidermal growth factor receptor. The present invention also relates to a composition comprising the antibody-drug conjugate, and use of the antibody-drug conjugate in manufacture of a medicament for the prophylaxis and/or treatment of a disease associated with epidermal growth factor receptor, in particular in manufacture of a medicament for prophylaxis and/or treatment of colon cancer, rectal cancer, head and neck cancer, lung cancer, ovarian cancer, cervical cancer, bladder cancer and esophageal cancer. The antibody-drug conjugate of the invention has a good inhibition activity on tumor cell growth both *in vivo* and *in vitro*, and has low toxicity, and thus has a good application prospect.

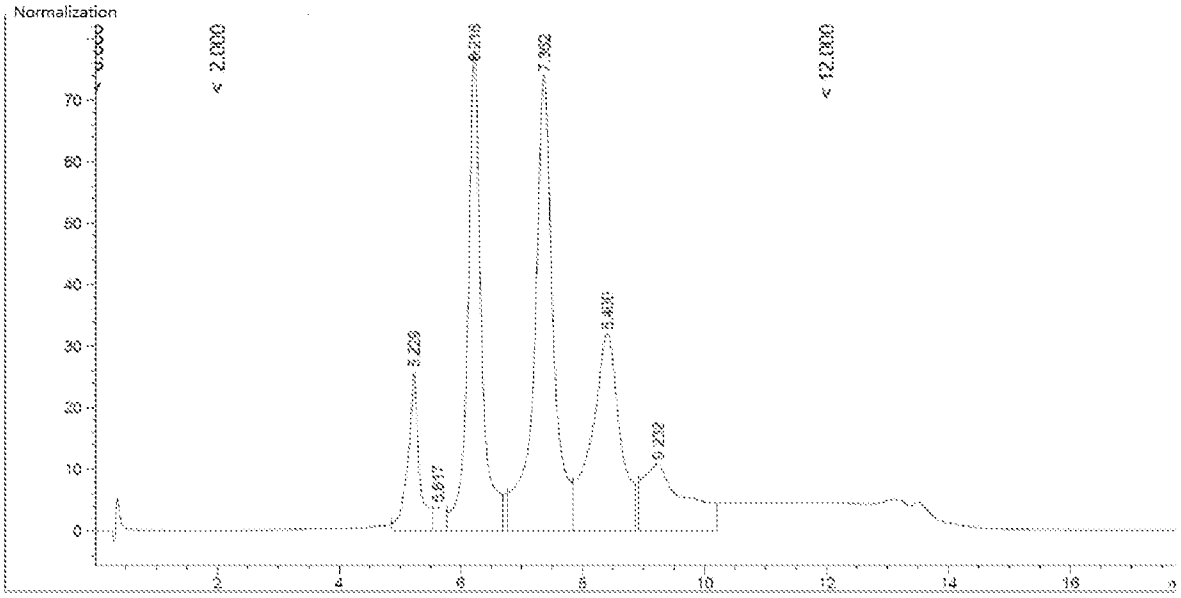


Fig.1

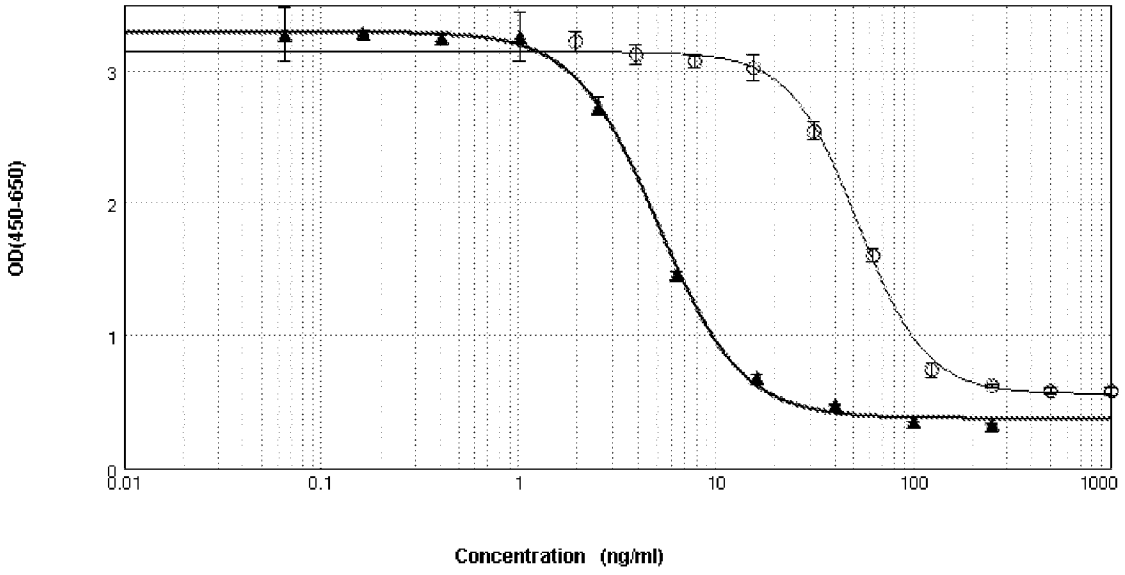


Fig.2

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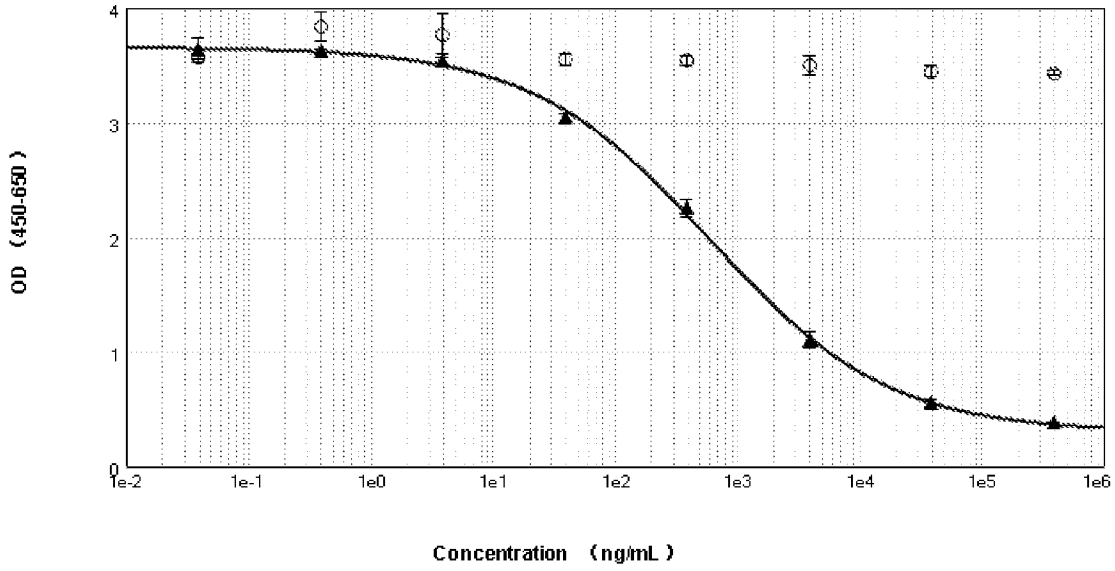

OD (450-650)
4
3
2
1
0
1e-2
1e-1
1e0
1e1
1e2
1e3
1e4
1e5
1e6
Concentration (ng/mL)

Fig.3

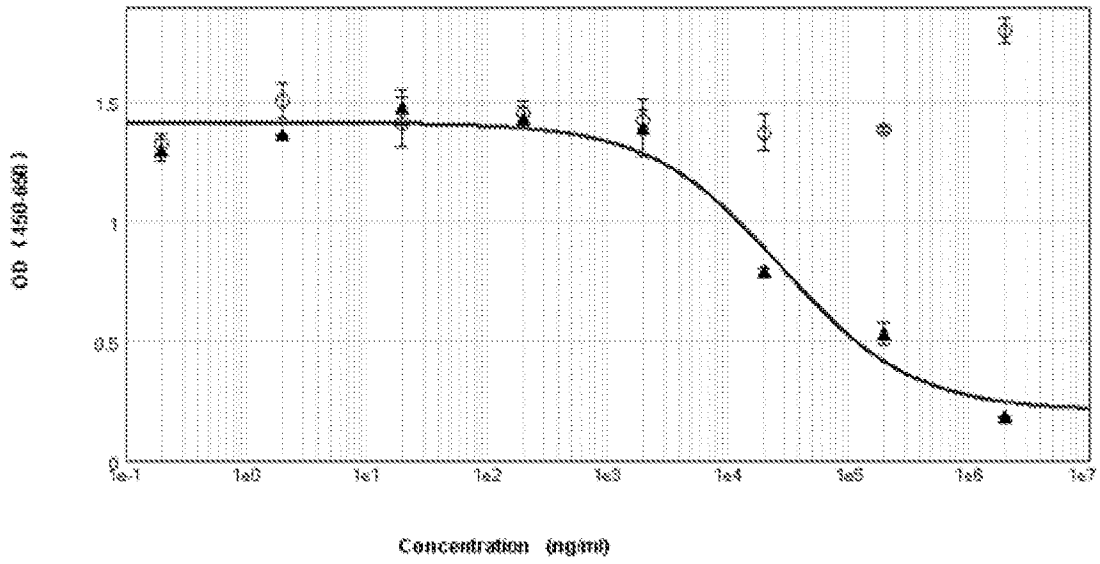

OD (450-650)
1.5
1
0.5
0
Concentration (ng/ml)

Fig.4

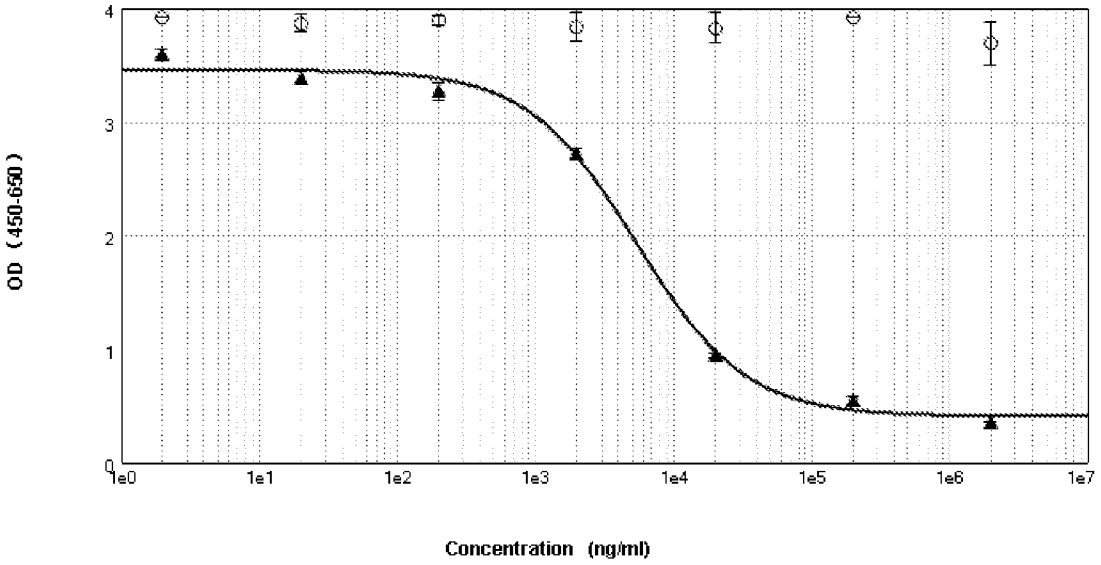

OD (450-650)
4
3
2
1
0
1e0
1e1
1e2
1e3
1e4
1e5
1e6
1e7
Concentration (ng/ml)

Fig.5

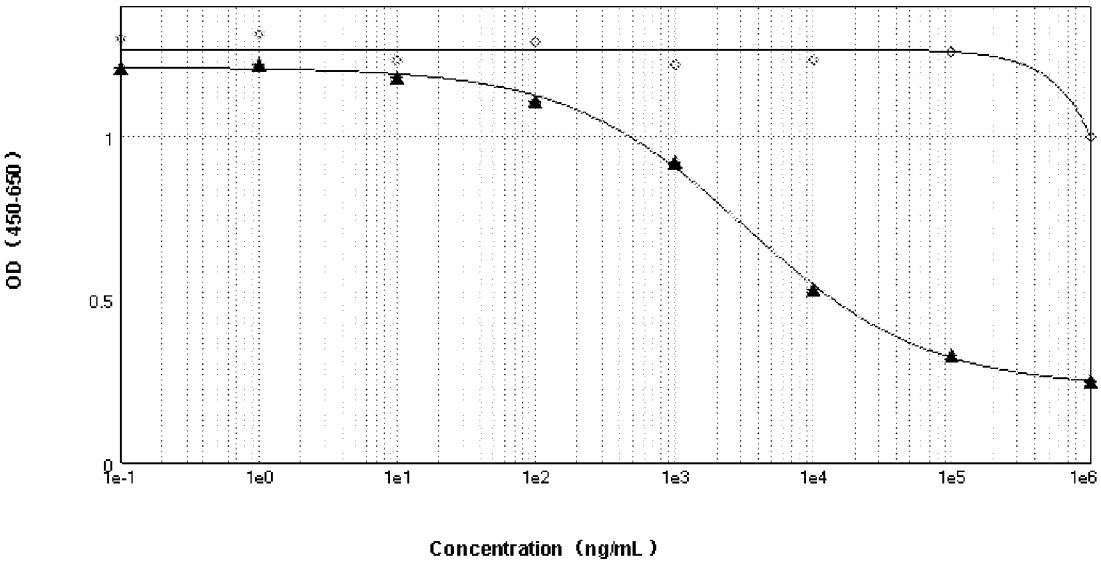

OD (450-650)
1
0.5
0
1e-1
1e0
1e1
1e2
1e3
1e4
1e5
1e6
Concentration (ng/mL)

Fig.6

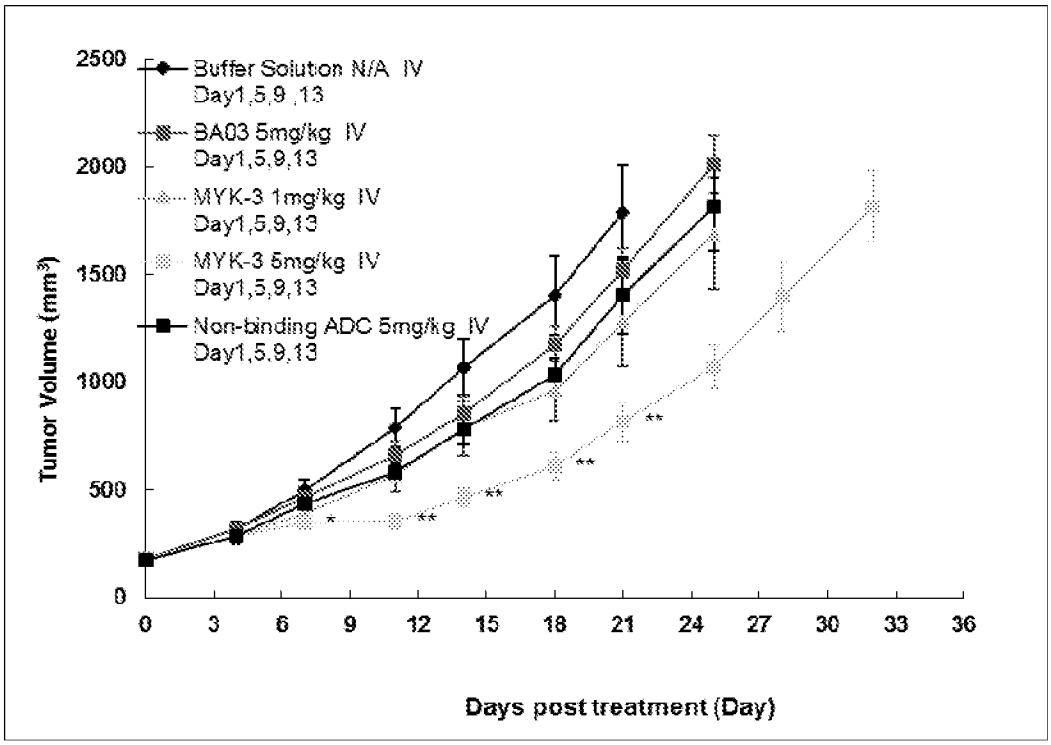


Fig.7

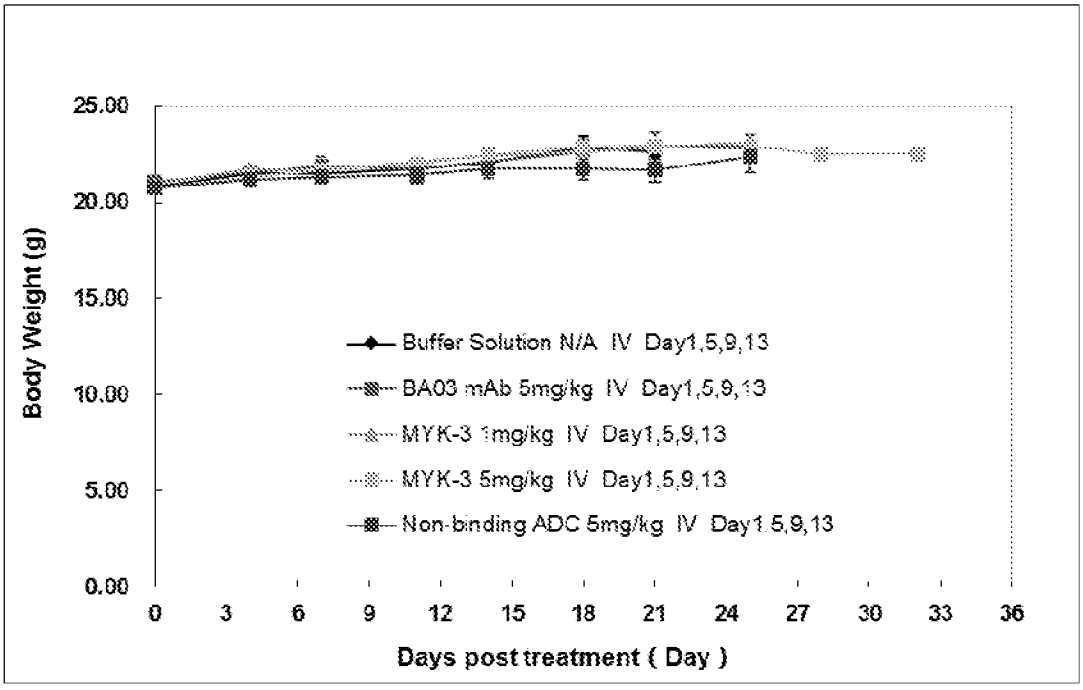


Fig.8

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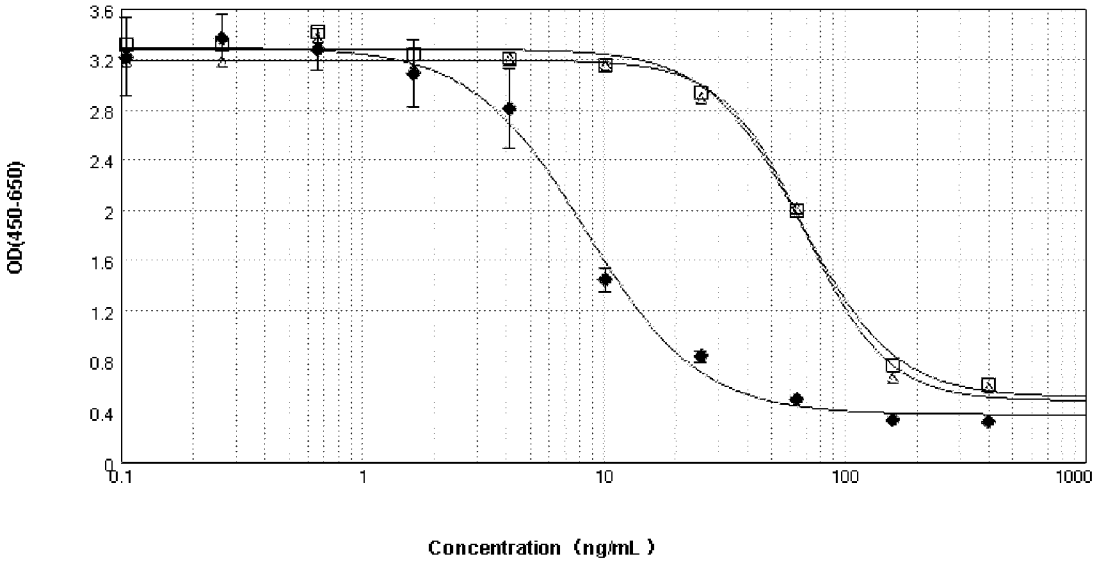


Fig.9

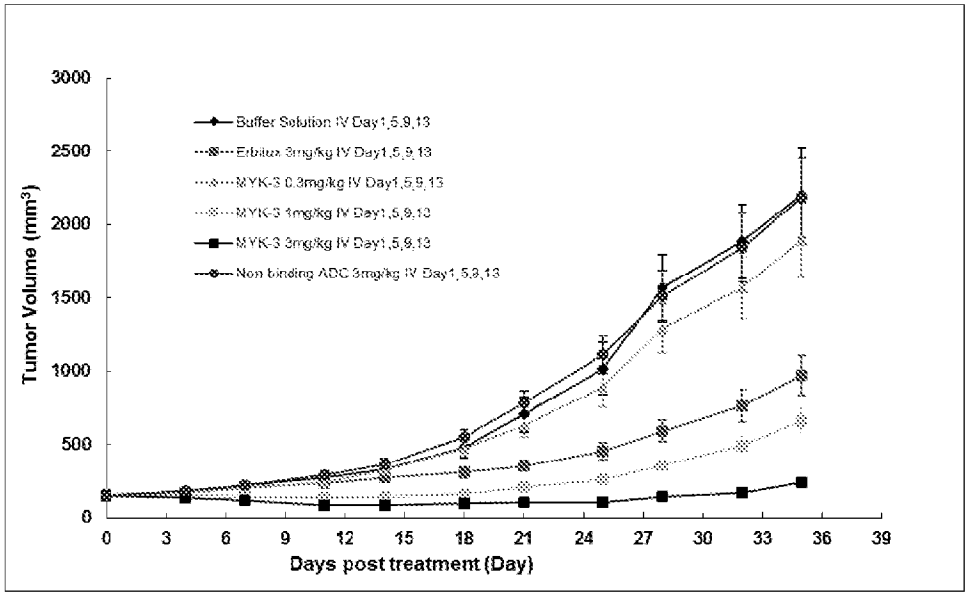


Fig.10

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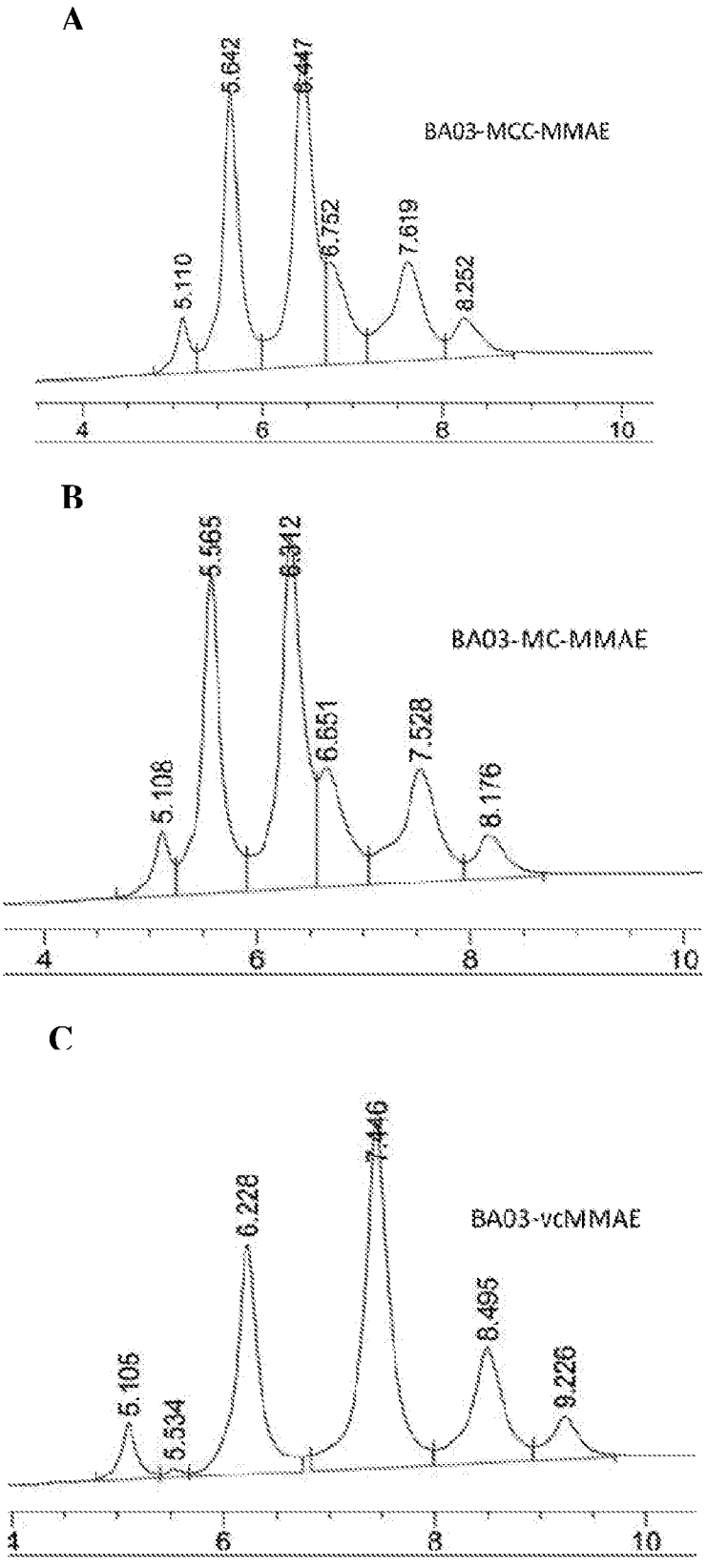


Fig.11

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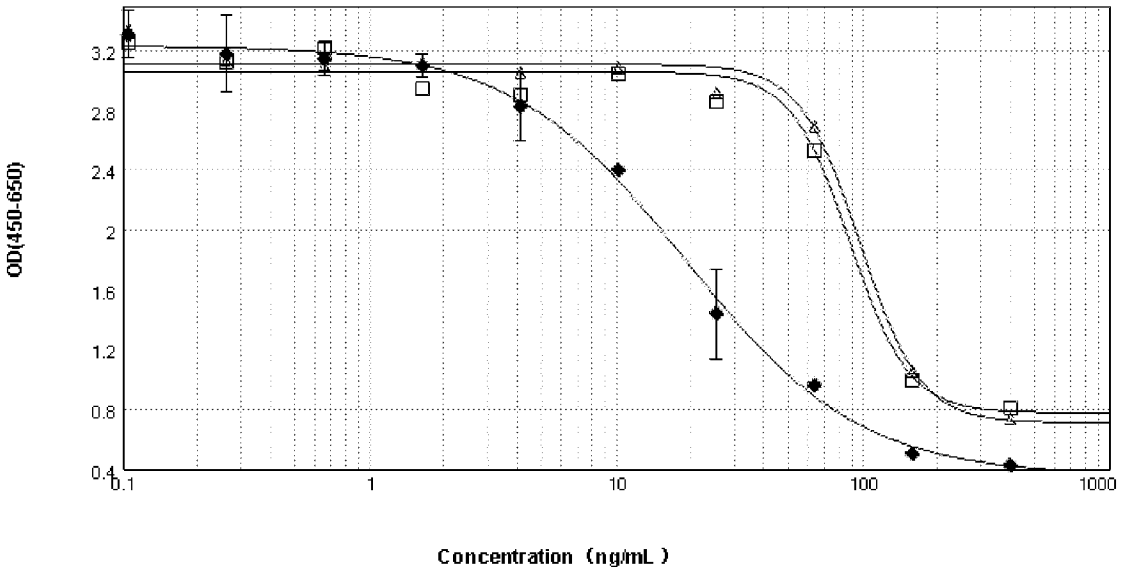

OD(450-650)
3.2
2.8
2.4
2
1.6
1.2
0.8
0.4
0.1
1
10
100
1000
Concentration (ng/mL)

Fig.12

(12) 按照专利合作条约所公布的国际申请

(19) 世界知识产权组织
国 际 局

(43) 国际公布日
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(81) 指定国 (除另有指明，要求每一种可提供的国家保护): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW。

(84) 指定国 (除另有指明，要求每一种可提供的地区保护): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), 欧亚 (AM, AZ, BY, KG, KZ, RU, TJ, TM), 欧洲 (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG)。

根据细则 4.17 的声明:
— 发明人资格(细则 4.17(iv))

本国际公布:
— 包括国际检索报告(条约第 21 条(3))。
— 包括说明书序列表部分(细则 5.2(a))。

(54) Title: ANTIBODY-DRUG CONJUGATE

(54) 发明名称 : 抗体药物偶联物

(57) Abstract: Provided is an antibody-drug conjugate targeting an epidermal growth factor receptor (EGFR), and also disclosed are a composition comprising the antibody-drug conjugate and a use of the antibody-drug conjugate in the preparation of a drug for preventing and/or treating diseases associated with the epidermal growth factor receptor.

(57) 摘要: 提供了一种抗表皮生长因子受体抗体药物偶联物，还公开了包含该抗体药物偶联物的组合物和该抗体药物偶联物在制备预防和/或治疗与表皮生长因子受体有关的疾病的药物中的应用。

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抗体药物偶联物

技术领域

本发明涉及抗体药物偶联物，具体涉及抗表皮生长因子受体抗体药物偶联物。本发明还涉及包含该抗体药物偶联物的组合物，以及该抗体药物偶联物的医药用途。

背景技术

表皮生长因子受体 (Epidermal Growth Factor Receptor，EGFR，也称为 HER1， c-ErbB1) 是表皮生长因子家族的细胞表面受体，是由 1186 个氨基酸残基构成，分子量为 170kD 的一种跨膜糖蛋白 (Jorissen RN，Walker F，Pouliot N，et al.Epidermal growth factor receptor：mechanisms of activation and signaling.Exp Cell Res，2003；284：31-53)。EGFR 属于Ⅰ型酪氨酸激酶受体 ErbB 亚族 (ErbB 1-4)，具有酪氨酸激酶的活性。EGFR 稳定的表达于许多上皮组织，包括皮肤和毛囊。表皮生长因子受体异常表达或因受体突变而活化会导致癌变。有很多实体瘤发现过度表达表皮生长因子受体，如结直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌和食管癌等 (Olayioye MA，Neve RM，Lane HA，et al.TheEerbB Signaling network：receptor heterodimerzation in development and cancer.The EMBO J，2000；19：3159-3167)。转化生长因子 α 和表皮生长因子等生长因子是表皮生长因子受体的内源性配体。这些配体与表皮生长因子受体结合，激活受体胞内酪氨酸蛋白激酶的活性，启动下游多条信号转导途径，从而调节正常细胞的生长和分化，增加肿瘤细胞的侵袭力、促进血管生成、抑制肿瘤细胞的凋亡 (Ciardiello F，Tortora G.A novel approach in the treatment of cancer：targeting the epidermal growth factor receptor.Clin Cancer Res，2001；7：2958-2970)。表皮生长因子受体在肿瘤中的过表达及其在肿瘤细胞生长、分化中起重要作用的这些特点，使表皮生长因子受体成为具有良好前景的肿瘤治疗靶点。

目前市场上已有两个抗表皮生长因子受体的治疗性抗体，一个是人鼠嵌合抗体 C225 抗体 (爱必妥，Erbitux 或 Cetuximab，ImClone(现在 Eli Lilly) 公司)，与表皮生长因子受体有特异亲和性，可阻断 EGF 和 TGFα 等配体与表皮生长因子受体的结合，抑制其磷酸化和下游信号传导，从而抑制肿瘤细胞的生长，诱导凋亡，减少基质金属蛋白酶和血管上皮生长因子的

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产生。2004 年美国 FDA 批准爱必妥治疗结直肠癌，2006 年批准其治疗头颈部癌，目前有更多临床试验用于其它肿瘤适应症。在临床上，爱必妥和伊立替康合用治疗结直肠癌的有效率（ORR）为 23%，与氟嘧啶等化疗药物合用治疗头颈癌的有效率为 13%-30%。 由于是人鼠嵌合抗体，爱必妥在临床试验中有 3.7%的病人产生了抗体反应。

另一个抗表皮生长因子受体的治疗性抗体是帕尼单抗 (Vectibix, panitumumab，Amgen 公司)， 是采用转基因小鼠技术制备的全人化单克隆抗体，无鼠源蛋白序列。该抗体靶向作用于表皮生长因子受体 (EGFR)，2006 年 9 月被 FDA 批准上市，与氟嘧啶、奥沙利铂和伊立替康合用或在化疗后用于治疗 EGFR 阳性的转移性结直肠癌。2006 年 FDA 批准其单药治疗化疗耐受的转移性结直肠癌(mCRC)。然而帕尼单抗是 IgG2 亚型抗体，与 IgG1 相比，IgG2 的 CDC 活性及 ADCC 等生物学活性明显减低；另外，IgG2 亚型抗体稳定性较差，这可能是临床效果上全人抗体帕尼单抗与嵌合抗体爱必妥相比没有明显优势的主要原因。在临床上治疗结直肠癌的总体生存率（OR）才达到 8%，非进展生存期只延长了 3.6 个月。

目前大量的临床数据显示爱必妥单抗和帕尼单抗只对 EGFR 表达的 KRAS 野生型 （KRAS wild type）有疗效，而对 KRAS 突变体没有肿瘤生长抑制活性，因此， 美国临床肿瘤协会发表的指导原则中明确指出抗 EGFR 单抗药物只适用于 KRAS 野生型的结肠癌病人 （Allegra CJ, Jessup JM, Somerield MR, Hamilton SR, Hammond EH, Hayes DF, et al. American Soceitey of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor monoclonal antibody therapy. J. Clin Oncol. 2009; 27:2091-2096; Bardelli A, Siena S. Molecular mechanisms of resistance to cetuximab and panitumumab in colorectal cancer. J Clin Oncol. 2010; 28:1254-1261）。

因此，本领域亟需具有更高生物学活性的人源化抗表皮生长因子受体的抗体类药物，尤其是对 KRAS 突变体有疗效的抗体类药物，例如抗体药物偶联物，以进一步提高疗效，降低副作用。

由于单抗癌症药物的引领，近年来在欧美快速发展的抗体药物偶联物（antibody drug conjugate， ADC）成为目前最前沿的医药技术之一。

抗体药物偶联物一般由三个部分组成:

1. 专一性结合靶点的单抗;

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2. 具有细胞毒性的小分子化学药物;

3. 链接小分子药物和单抗的链接体，也叫接头。

抗体药物偶联物利用单抗的靶点专一性和化学药物的细胞毒性来杀死肿瘤细胞。它的作用机制是：（1）抗体药物偶联物利用单抗特异地结合到肿瘤细胞上的靶点抗原；（2）抗体药物偶联物和靶点抗原的复合物通过靶点抗原介导内吞进入细胞；（3）抗体药物偶联物在细胞内降解，释放具有细胞毒的化学药物；（4）具有细胞毒的化学药物杀死肿瘤细胞。

抗体药物偶联物的作用机制看似简单，但是一个抗体药物偶联物是否能成为安全有效的药物是非常复杂和不可预测的，依赖多种因素，例如：

1) 靶点的特性： 靶点是否能内吞噬 （internalize），靶点的表达水平，靶点是否在癌细胞和正常细胞中有足够的表达水平差异，靶点是否会脱落细胞外部分（extracellular domain, ECD）到血液中；

2) 单抗的特性： 单抗对靶点的特异性是否足够好 （与其它蛋白无交叉反应），单抗的稳定性如何，单抗和靶点的复合物是否能内吞噬入细胞；

3) 小分子化学药物的特性： 小分子化学药物的细胞毒性是否足够强，其在血液里的稳定性，以及其成为 ADC 后在体内的代谢产物的毒性；

4) 接头的特性： 接头是可切割的 （cleavable）还是不可切割的 (non-cleavable)，接头在血液里的稳定性；

5) ADC 的特性：ADC 和靶点的复合物是否能内吞噬，ADC 在血液里的稳定性，ADC 上用什么链接体和链接几个化学药物，ADC 杀死癌细胞的活性和生物毒性。

可以看出， ADC 药物的研发需要大量的实验摸索和验证，其安全性和有效性是无法在实验前预测的。

发明内容

本发明的发明人通过大量实验和创造性劳动，制备得到了抗表皮生长因子受体抗体药物偶联物，并证实其具有良好的生物学活性，由此完成了本发明。

本发明第一方面涉及抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其包含与细胞毒剂共价连接的抗表皮生长因子受体抗体。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体包括重链和轻链，其中重链可变区 CDR1、CDR2、CDR3 分别包含如 SEQ ID

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NO: 5～7所示的序列或其突变体，轻链可变区CDR1、CDR2、CDR3分别包含如SEQ ID NO: 12～14所示的序列或其突变体。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体轻链可变区CDR1、CDR2、CDR3区分别包含如SEQ ID NO: 12～14所示的序列或者与SEQ ID NO: 12～14所示的序列的同一性大于70%，例如大于75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%的序列，例如具有3个、2个或1个突变、缺失或添加氨基酸的序列。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体重链可变区CDR1、CDR2、CDR3区分别包含如SEQ ID NO: 5～7所示的序列或者与SEQ ID NO: 5～7所示的序列的同一性大于70%，例如大于75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%的序列，例如具有3个、2个或1个突变、缺失或添加氨基酸的序列。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体重链可变区FR1、FR2、FR3、FR4区分别包含如SEQ ID NO: 8～11所示的序列或其突变体。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体重链可变区FR1、FR2、FR3、FR4区分别包含如SEQ ID NO: 8～11所示的序列，或者包含与上述序列的同一性大于70%，例如大于75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%的序列。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体轻链可变区FR1、FR2、FR3、FR4区分别包含如SEQ ID NO: 15～18所示的序列或其突变体。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体轻链可变区FR1、FR2、FR3、FR4区分别包含如SEQ ID NO: 15～18所示的序列，或者包含与上述序列的同一性大于70%，例如大于75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%的序列。

在本发明的一个实施方案中，所述抗表皮生长因子受体抗体的重链可变区的序列如SEQ ID NO: 1所示。

在本发明的一个实施方案中，所述抗表皮生长因子受体抗体的轻链可

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变区的序列如 SEQ ID NO: 2 所示。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体的重链恒定区选自人源性 IgG、IgM、IgA、IgD、IgA 恒定区或上述恒定区的突变体。

在本发明的一个实施方案中，其中所述 IgG 选自 IgG1、IgG2、IgG3 和 IgG4。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体重链恒定区的氨基酸序列包含如 SEQ ID NO: 3 所示的序列，或者包含与 SEQ ID NO: 3 所示序列的同一性大于 70%，例如大于 75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%的序列。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体的轻链恒定区为人源性 lambda 恒定区、kappa 恒定区、或上述恒定区的突变体。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体轻链恒定区的氨基酸序列包含如 SEQ ID NO: 4 所示的序列，或者包含与 SEQ ID NO: 4 所示序列的同一性大于 70%，例如大于 75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%的序列。

在本发明的一个实施方案中，其中所述的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物具有式 I 所示的结构，

$$Ab\text{-}(L\text{-}D)_p$$

式 I

其中：

Ab 代表抗表皮生长因子受体抗体；

L 代表接头；

D 代表细胞毒剂；

p 代表 1-8，例如 2-6，例如 3-5。

在本发明的一个实施方案中，其中所述的细胞毒剂选自化疗药物、毒素（例如细菌、真菌、植物、或动物源性的酶活性毒素或其片段）、放射性同位素、细胞因子、抗生素、酶、纳米颗粒和生物活性肽。

在本发明的一个实施方案中，其中所述的细胞毒剂选自 Monomethyl auristatin E（MMAE）、 Monomethyl auristatin F（MMAF）、 美登木素生物碱(例如Maytansine DM1、Maytansine DM4)、卡奇霉素(calicheamicin)、duocarmycin MGBA、阿霉素(doxorubicin)、蓖麻毒素、白喉毒素等毒素、

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I131、白介素类、肿瘤坏死因子、趋化因子和纳米颗粒等。

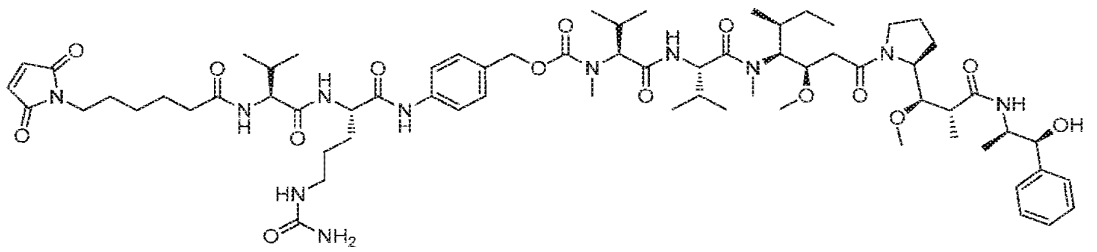
在本发明的一个实施方案中，其中所述的细胞毒剂为MMAE。

在本发明的一个实施方案中，其中所述的接头为可切割的或不可切割的。

在本发明的一个实施方案中，其中所述的接头选自 6-马来酰亚氨基己酰基(MC)、马来酰亚氨基丙酰基(MP)、缬氨酸-瓜氨酸(val-cit)、丙氨酸-苯丙氨酸(ala-phe)、对氨基苄氧羰基(PAB)、N-琥珀酰亚氨基 4-(2-吡啶基硫代)戊酸酯(SPP)、N-琥珀酰亚氨基 4-(N-马来酰亚氨基甲基)-环己烷-1-羧酸酯(SMCC)、N-琥珀酰亚氨基(4-碘-乙酰基)氨基苯甲酸酯(SIAB)、和 6-马来酰亚氨基己酰基-缬氨酸-瓜氨酸-对氨基苄氧羰基(MC-vc-PAB)。

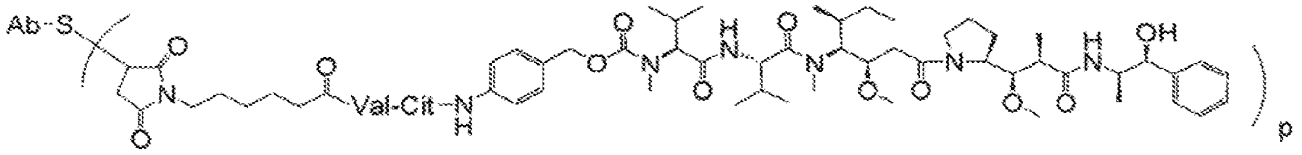
在本发明的一个实施方案中，其中所述的接头为 6-马来酰亚氨基己酰基-缬氨酸-瓜氨酸-对氨基苄氧羰基(MC-vc-PAB)。

在本发明的一个实施方案中，其中式 I 中所述的 L-D 为 vc-MMAE，其结构如下式所示：



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在本发明的一个具体实施方案中，其中所述的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物如下式所示：



其中Ab为抗表皮生长因子受体抗体，p为1-8，例如2-6，例如3-5。

本发明第二方面涉及组合物（例如药物组合物），其含有本发明第一方面任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，任选地，还含有至少一种药学上可接受的载体、稀释剂或赋形剂。

在本发明的一个实施方案中，所述组合物中还包含已知的用于治疗肿瘤

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的化疗药物，所述化疗药物例如为阿霉素(Adriamycin)、环磷酰胺和紫杉烷类[紫杉醇(Taxol)和多西他赛(Taxotere)]、卡培他滨(Xeloda)、吉西他滨(Gemzar)、长春瑞滨(Navelbine)、他莫昔芬、芳香酶抑制剂(瑞宁得、弗隆、阿诺新)、5-FU 加亚叶酸、伊立替康(camptosar)、奥沙利铂、顺铂、卡铂、蒽莫司汀、米托蒽醌(Novantrone)、泼尼松、长春新碱(Oncovin)等，或它们的组合。

本发明还涉及本发明第一方面任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物在制备预防和/或治疗与表面生长因子受体（EGFR）相关的疾病的药物中的用途。

在本发明的一个实施方案中，其中所述的与表面生长因子受体（EGFR）相关的疾病为与EGFR相关的肿瘤，例如与EGFR过表达相关的肿瘤，例如选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

在本发明一个实施方案中，其中所述肿瘤为KRAS基因突变的肿瘤，例如为KRAS基因突变的结肠癌、直肠癌、肺癌或胰腺癌。

在本发明一个实施方案中，其中所述的肿瘤为BRAF基因突变的肿瘤，例如选自BRAF基因突变的结肠癌、直肠癌和肺癌。

本发明还涉及本发明第一方面任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物在制备抑制肿瘤血管生成、延缓肿瘤进展、抑制肿瘤生长、抑制肿瘤细胞增殖的药物中的用途。

在本发明的一个实施方案中，其中所述的肿瘤选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

在本发明一个实施方案中，其中所述肿瘤为KRAS基因突变的肿瘤，例如为KRAS基因突变的结肠癌、直肠癌、肺癌或胰腺癌。

在本发明一个实施方案中，其中所述的肿瘤为BRAF基因突变的肿瘤，例如选自BRAF基因突变的结肠癌、直肠癌和肺癌。

本发明还涉及预防和/或治疗与表皮生长因子受体（EGFR）相关的疾病的方法，所述方法包括给予有需要的受试者预防和/或治疗有效量的本发明第一方面任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述

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盐的溶剂合物。

在本发明的一个实施方案中，其中所述的与表皮生长因子受体（EGFR）相关的疾病为与EGFR相关的肿瘤，例如与EGFR过表达相关的肿瘤，例如选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

在本发明一个实施方案中，其中所述肿瘤为KRAS基因突变的肿瘤，例如为KRAS基因突变的结肠癌、直肠癌、肺癌或胰腺癌。

在本发明一个实施方案中，其中所述的肿瘤为BRAF基因突变的肿瘤，例如选自BRAF基因突变的结肠癌、直肠癌和肺癌。

本发明还涉及抑制肿瘤血管生成、延缓肿瘤进展、抑制肿瘤生长、抑制肿瘤细胞增殖的方法，所述方法包括给予有需要的受试者预防和/或治疗有效量的本发明第一方面任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物。

在本发明的一个实施方案中，其中所述的肿瘤选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

在本发明一个实施方案中，其中所述肿瘤为KRAS基因突变的肿瘤，例如为KRAS基因突变的结肠癌、直肠癌、肺癌或胰腺癌。

在本发明一个实施方案中，其中所述的肿瘤为BRAF基因突变的肿瘤，例如选自BRAF基因突变的结肠癌、直肠癌和肺癌。

本发明还涉及本发明第一方面任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其用于预防和/或治疗与表皮生长因子受体（EGFR）相关的疾病。

在本发明的一个实施方案中，其中所述的与表皮生长因子受体（EGFR）相关的疾病为与EGFR相关的肿瘤，例如与EGFR过表达相关的肿瘤，例如选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

在本发明一个实施方案中，其中所述肿瘤为KRAS基因突变的肿瘤，例如为KRAS基因突变的结肠癌、直肠癌、肺癌或胰腺癌。

在本发明一个实施方案中，其中所述的肿瘤为BRAF基因突变的肿瘤，例如选自BRAF基因突变的结肠癌、直肠癌和肺癌。

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本发明还涉及本发明第一方面任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其用于抑制肿瘤血管生成、延缓肿瘤进展、抑制肿瘤生长、抑制肿瘤细胞增殖。

在本发明的一个实施方案中，其中所述的肿瘤选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

在本发明一个实施方案中，其中所述肿瘤为KRAS基因突变的肿瘤，例如为KRAS基因突变的结肠癌、直肠癌、肺癌或胰腺癌。

在本发明一个实施方案中，其中所述的肿瘤为BRAF基因突变的肿瘤，例如选自BRAF基因突变的结肠癌、直肠癌和肺癌。

本发明的抗表皮生长因子受体抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物在体内和体外都具有良好的抑制肿瘤细胞生长活性，特别是对 EGFR 中度和低度表达的肿瘤细胞也显示出明显的细胞生长抑制活性，且细胞毒性低；更特别地，本发明的抗表皮生长因子受体抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物对 KRAS 基因突变或 BRAF 基因突变的肿瘤也具有很好的疗效，因而具有良好的应用前景。

以下对本发明做进一步描述:

在本发明中，除非另有说明，否则本文中使用的科学和技术名词具有本领域技术人员所通常理解的含义。并且，本文中所用的蛋白质和核酸化学、分子生物学、细胞和组织培养、微生物学、免疫学相关术语和实验室操作步骤均为相应领域内广泛使用的术语和常规步骤。同时，为了更好地理解本发明，下面提供相关术语的定义和解释。

在本发明中，术语“抗体”是指通常由两对相同的多肽链（每对具有一条“轻”（L）链和一条“重”（H）链）组成的免疫球蛋白分子。抗体的轻链可分为 κ 和 λ 两类。重链可分为 μ、δ、γ、α 或 ε 五种，依据重链的不同可将抗体分为 IgM、IgD、IgG、IgA 和 IgE 五类。在轻链和重链内，可变区和恒定区通过大约 12 或更多个氨基酸的“J”区连接，重链还包含大约 3 个或更多个氨基酸的“D”区。各重链由重链可变区(V_H)和重链恒定区(C_H)组成。重链恒定区由 3 个结构域(C_H1、C_H2 和 C_H3)组成。各轻链由轻链可变区(V_L)和轻链恒定区(C_L)组成。轻链恒定区由一个结构域 C_L 组成。抗体的

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恒定区可介导免疫球蛋白与宿主组织或因子，包括免疫系统的各种细胞(例如，效应细胞)和补体系统的组分 C1q 的结合。V_H 和 V_L 区还可被细分为具有高变性的区域(称为互补决定区(CDR))，其间散布有较保守的称为骨架区（FR）的区域。各 V_H 和 V_L 由按下列顺序：FR1、CDR1、FR2、CDR2、FR3、CDR3、FR4 从氨基末端至羧基末端排列的 3 个 CDR 和 4 个 FR 组成。各重链/轻链对的可变区(V_H 和 V_L)分别形成抗体结合部位。氨基酸至各区域或结构域的分配遵循 Kabat Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and 1991))，或 Chothia & Lesk (1987) J. Mol. Biol. 196:901-917; Chothia 等人 (1989) Nature 342:878-883 的定义。术语“抗体”不受任何特定的产生抗体的方法限制。例如，其包括，特别地，重组抗体、单克隆抗体和多克隆抗体。抗体可以是不同类型的抗体，例如，IgG (例如，IgG1，IgG2，IgG3 或 IgG4 亚型)，IgA1，IgA2，IgD，IgE 或 IgM 抗体。

在本发明中，所述 IgG 重链恒定区包括 IgG1、 IgG2、IgG3 或 IgG4。在本发明的实施方案中，所述 IgG 重链恒定区为 IgG1 型。

在本发明中，所述 κ 轻链恒定区包括各种同种异型，如 Km1、 Km1,2 或 Km3。

关于抗体的氨基酸序列

在本发明的实施方案中，抗表皮生长因子受体抗体的重链可变区氨基酸序列是 SEQ ID NO:1。在本发明的实施方案中，抗表皮生长因子受体抗体的轻链可变区氨基酸序列是 SEQ ID NO:2。

另一方面，本发明所述的抗体的重链可变区的氨基酸序列与 SEQ ID NO:1 的序列相似性至少达 70%，优选是至少 75%，优选是至少 80%，优选是 85%，再优选是至少 90%，最好的是至少 95%。

另一方面，本发明所述的抗体的轻链可变区的氨基酸序列与 SEQ ID NO:2 的序列相似性至少达 70%，优选是至少 75%，优选是至少 80%，优选是 85%，再优选是至少 90%，最好的是至少 95%。

在本发明的实施方案中，抗表皮生长因子受体抗体的重链和轻链可变区的 CDR 的氨基酸序列确定如下：

重链的 CDR1、CDR2 和 CDR3 的氨基酸序列分别为 SEQ ID NO: 5-7；轻链的 CDR1、CDR2 和 CDR3 的氨基酸序列分别 SEQ ID NO: 12-14。

另一方面，抗表皮生长因子受体抗体的重链的 CDR 含有的氨基酸序列可能是在 SEQ ID NO: 5-7 上出现一个或多个氨基酸的突变或增添或缺失。

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优选的是，突变、添加或缺失的氨基酸不超过 3 个氨基酸。更优选的是，突变、添加或缺失的氨基酸不超过 2 个氨基酸。最优选的是，突变、添加或缺失的氨基酸不超过 1 个氨基酸。

另一方面，抗表皮生长因子受体抗体的轻链的 CDR 含有的氨基酸序列可能是在 SEQ ID NO: 12-14 上出现一个或多个氨基酸的突变、增添或缺失。优选的是，突变、添加或缺失的氨基酸不超过 3 个氨基酸。更优选的是，突变、添加或缺失的氨基酸不超过 2 个氨基酸。最优选的是，突变、添加或缺失的氨基酸不超过 1 个氨基酸。

在本发明的实施方案中，抗表皮生长因子受体抗体的重链和轻链可变区的 FR 的氨基酸序列确定如下：

重链可变区 FR1、FR2、FR3、FR4 的序列分别为 SEQ ID NO: 8-11。轻链可变区 FR1、FR2、FR3、FR4 的序列分别为 SEQ ID NO: 15-18。

另一方面，抗表皮生长因子受体抗体的重链或轻链可变区 FR 的氨基酸序列可能是在 SEQ ID NO: 8-11、SEQ ID NO: 15-18 上出现一个或多个氨基酸的突变、增添或缺失。优选的是，突变、添加或缺失的氨基酸不超过 3 个氨基酸。更优选的是，突变、添加或缺失的氨基酸不超过 2 个氨基酸。最优选的是，突变、添加或缺失的氨基酸不超过 1 个氨基酸。

上述抗体或 CDR 区或框架区的氨基酸发生突变、添加或缺失之后的变异体仍然保留特异性结合 EGFR 的能力。

在本发明的实施方案中，抗表皮生长因子受体抗体重链恒定区氨基酸序列是 SEQ ID NO:3。在本发明的实施方案中，抗表皮生长因子受体抗体的轻链恒定区氨基酸序列是 SEQ ID NO:4。

另一方面，本发明所述的抗体的重链恒定区的氨基酸序列与 SEQ ID NO:3 的序列相似性至少达 70%，优选是至少 75%，优选是至少 80%，优选是 85%，再优选是至少 90%，最好的是至少 95%，如 96%、97%、98%、99%。

另一方面，本发明所述的抗体的轻链恒定区的氨基酸序列与 SEQ ID NO:4 的序列相似性至少达 70%，优选是至少 75%，优选是至少 80%，优选是 85%，再优选是至少 90%，最好的是至少 95%，如 96%、97%、98%、99%。

本发明所述的单抗变异体可以通过传统的基因工程方法获得。本领域的技术人员完全知晓利用核酸突变改造 DNA 分子的方法。另外，编码重链和轻链变异体的核酸分子也可以通过化学合成获得。

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在本发明中，用于确定序列同一性（同源性）和序列相似性百分数的算法是例如 BLAST 和 BLAST 2.0 算法，它们分别描述在 Altschul 等（1977）Nucl. Acid. Res. 25: 3389-3402 和 Altschul 等（1990）J. Mol. Biol. 215: 403-410。采用例如文献中所述或者默认参数，BLAST 和 BLAST 2.0 可以用于确定本发明的氨基酸序列同一性百分数。执行 BLAST 分析的软件可以通过国立生物技术信息中心为公众所获得。

在本发明中，所述与氨基酸序列具有至少 70％的序列同一性的氨基酸序列包括与所述氨基酸序列基本同一的多肽序列，例如当采用本文所述方法（例如采用标准参数的 BLAST 分析）时，与本发明多肽序列相比含有至少 70％序列同一性、优选至少 75％、80％、85％、86％、87％、88％、89％、90％、91%、92%、93%、94%、95%、96%、97%、98%、99％或更高的序列同一性的那些序列。

在本发明中，用于抗体药物偶联物的毒素包括白喉毒素 A 链、白喉毒素的非结合活性片段、外毒素 A 链（来自铜绿假单胞菌(Pseudomonas aeruginosa)）、蓖麻毒蛋白(ricin)A 链、相思豆毒蛋白(abrin)A 链、蒴莲根毒蛋白(modeccin)A 链、α-帚曲霉素(sarcin)、油桐(Aleutites fordii)毒蛋白、香石竹(dianthin)毒蛋白、美洲商陆(Phytolacaamericana)毒蛋白(PAPI、PAPII 和 PAP-S)、苦瓜(Momordica charantia)抑制物、麻疯树毒蛋白(curcin)、巴豆毒蛋白(crotin)、肥皂草(sapaonaria officinalis)抑制物、白树毒蛋白(gelonin)、丝林霉素(mitogellin)、局限曲菌素(restrictocin)、酚霉素(phenomycin)、依诺霉素(enomycin)、和单端孢菌素(trichothecenes)。参见例如 1993 年 10 月 28 日公布的 WO 93/21232。

多种放射性核素可用于生成抗体药物偶联物。例子包括 ^{212}Bi、^{131}I、^{131}In、^{90}Y、和 ^{186}Re。

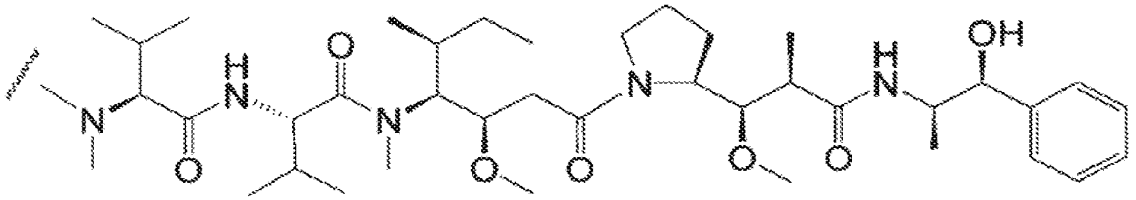
抗体和细胞毒剂的偶联物可使用多种双功能蛋白质偶联剂来制备，诸如 N-琥珀酰亚氨基 3-(2-吡啶基二硫代)丙酸酯(SPDP)，亚氨基硫烷(IT)，亚氨酸酯(诸如盐酸己二酰亚氨酸二甲酯)、活性酯类(诸如辛二酸二琥珀酰亚氨基酯)、醛类(诸如戊二醛)、双叠氮化合物(诸如双(对-叠氮苯甲酰基)己二胺)、双重氮衍生物(诸如双(对-重氮苯甲酰基)-乙二胺)、二异硫氰酸酯(诸如甲苯 2，6-二异氰酸酯)、和双活性氟化合物(诸如 1，5-二氟-2，4-二硝基苯)的双功能衍生物。例如，可如 Vitetta 等在(1987)Science，238: 1098 中所述制备的含蓖麻毒的免疫毒素。碳-14 标记的 1-异硫氰酸苄基-3-甲基二亚乙基三胺五乙

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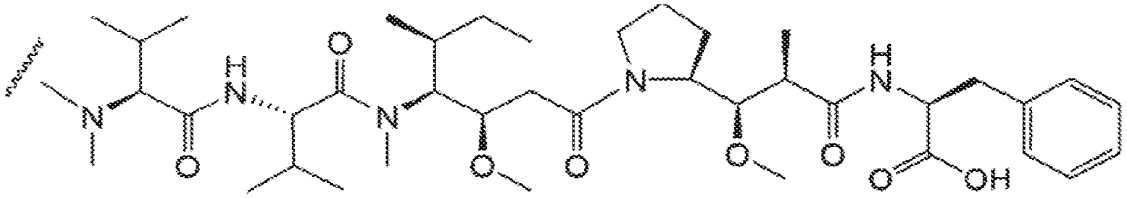
酸(MX-DTPA)是用于将放射性核苷酸与抗体偶联的例示性螯合剂(WO94/11026)。 本发明还涵盖抗体和一种或多种小分子毒素(诸如加利车霉素(calicheamicin)、美登素生物碱(maytansinoid)、多拉司他汀(dolastatin)、 auristatin、单端孢霉素(trichothecene)、和 CC 1065 及这些毒素的具有毒素活性的衍生物的偶联物。

在有些实施方案中，抗体药物偶联物包含与多拉司他汀(dolastatin)或多拉司他汀肽类似物和衍生物 auristatin(美国专利 No.5,635,483; 5,780,588)偶联的抗表皮生长因子受体抗体。多拉司他汀和 auristatin 已经显示出干扰微管动力学、GTP 水解、及核和细胞分裂等活性(Woyke 等(2001)Antimicrob.Agents and Chemother.45(12): 3580-3584)且具有抗癌(US 5,663,149)和抗真菌活性(Pettit 等(1998) Antimicrob.Agents Chemother.42: 2961-2965)。多拉司他汀或 auristatin 药物模块可经由肽药物模块的 N(氨基)末端或 C(羧基)末端附着于抗体(WO 02/088172)。

在本发明中，其中 MMAE 的结构为:



在本发明中，其中 MMAF 的结构为:



在本发明中，作为细胞毒剂的酶可以为具有核酸降解活性的化合物(例如核糖核酸酶或 DNA 内切核酸酶，诸如脱氧核糖核酸酶; DNA 酶)。

在本发明中，药物载荷(loading)由 p 表示，即式 I: Ab-(L-D)$_p$ 的分子中每个抗体的平均药物模块（即细胞毒剂）数。药物载荷的范围可以为每个抗体 1-20 个药物模块(D)。通式 I 的 ADC 包括偶联有一定范围(1-20 个)药物模块的抗体的集合。来自偶联反应的 ADC 制备物中每个抗体的平均药物模块数可以通过常规手段来验证，诸如质谱、ELISA 测定法、和 HPLC。还可以测定 ADC 在 p 方面的定量分布。在有些情况中，将 p 为某数值的同质 ADC

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从具有其它药物载荷的 ADC 中分离、纯化和验证可以通过诸如反相 HPLC 或电泳的手段来实现。

对于有些抗体-药物偶联物，p 可能受到抗体上附着位点数目的限制。例如，若附着位点是半胱氨酸硫醇，抗体可能只有一个或数个半胱氨酸硫醇基，或者可能只有一个或数个有足够反应性的硫醇基，可附着接头。在某些实施方案中，较高的药物载荷，例如 $p > 5$，可引起某些抗体-药物偶联物的聚集、不溶性、毒性、或丧失细胞通透性。

在某些实施方案中，本发明 ADC 的药物载荷范围为 1 到约 8；约 2 到约 6；约 3 到约 5；约 4 到约 5；约 3.5 到 4.5；约 4。事实上，对于某些 ADC 已经显示了每个抗体的药物模块的最佳比率可以为小于 8，可以为约 2 到约 5。参见 US2005-0238649A1(完整收入本文作为参考)。

在某些实施方案中，在偶联反应中少于理论最大值的药物模块偶联至抗体。抗体可包含例如赖氨酸残基，其不与药物-接头中间物或接头试剂起反应。一般而言，抗体不包含许多游离的和反应性的半胱氨酸硫醇基，其可连接药物模块；事实上，抗体中的大多数半胱氨酸硫醇基以二硫桥形式存在。在某些实施方案中，可以在部分或完全还原性条件下用还原剂诸如二硫苏糖醇(DTT)或三羰基乙基膦(TCEP)还原抗体以产生反应性半胱氨酸硫醇基。在某些实施方案中，将抗体置于变性条件以暴露反应性亲核基团，诸如赖氨酸或半胱氨酸。

ADC 的载荷(药物/抗体比率)可以以不同方式来控制，例如通过：(i)限制药物-接头中间物或接头试剂相对于抗体的摩尔数，(ii)限制偶联反应的时间或温度，(iii)半胱氨酸硫醇修饰的部分或限制还原性条件，(iv)通过重组技术对抗体的氨基酸序列进行工程改造，使得半胱氨酸残基的数目和位置为了控制接头-药物附着的数目和/或位置而进行改变。应当理解，若超过一个亲核基团与药物-接头中间物或者与接头试剂和接下来的药物模块试剂起反应，则所得产物是具有一个或多个药物模块附着于抗体的 ADC 化合物混合物。可以通过对抗体特异性和对药物特异性的双重 ELISA 抗体测定法自混合物计算每个抗体的平均药物数。混合物中的各种 ADC 分子可以通过质谱来鉴定，并通过 HPLC 来分离，例如疏水相互作用层析。在某些实施方案中，可以通过电泳或层析从偶联混合物中分离具有单一载荷值的同质 ADC。

在本发明中，抗体药物偶联物的药学上可接受的盐包括无机酸、羧酸和磺酸的酸加成盐，例如以下酸的盐：盐酸、氢溴酸、硫酸、磷酸、甲烷磺酸、乙烷磺酸、苯磺酸、甲苯磺酸、萘二磺酸、乙酸、三氟乙酸、丙酸、乳酸、

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酒石酸、苹果酸柠檬酸、富马酸、马来酸和苯甲酸。

本发明的抗体药物偶联物的药学上可接受的盐还包括常规碱的盐，例如（仅为示例性并优选）碱金属盐（例如钠盐和钾盐）、碱土金属盐（例如钙盐和镁盐）和衍生自氨或含有1-16个碳原子的有机胺的铵盐，所述有机胺例如（仅为示例性并优选）乙胺、二乙胺、三乙胺、乙基二异丙胺、单乙醇胺、二乙醇胺、三乙醇胺、二环己胺、二甲基氨基乙醇、普鲁卡因、二苯甲酰胺、N- 甲基哌啶、N- 甲基吗啉、精氨酸、赖氨酸和1，2- 乙二胺。

在本发明中，溶剂合物表示这些形式的本发明的抗体药物偶联物：所述抗体药物偶联物通过与溶剂分子配位而形成的固态或液态形式的复合物。水合物是溶剂合物的一种具体形式，其具有配位的水分子。在本发明中，水合物是优选的溶剂合物。

可以优选地使用本发明的抗体药物偶联物进行治疗的与EGFR相关的肿瘤包括过表达EGFR 的肿瘤、呼吸道肿瘤（例如小细胞癌和非小细胞癌、支气管癌），其中特别优选非小细胞肺癌；消化道（例如食道、胃、胆囊、小肠、大肠、直肠）的肿瘤，其中特别优选肠肿瘤；内分泌腺和外分泌腺（例如甲状腺和甲状旁腺、胰腺和唾液腺）的肿瘤，其中特别优选胰腺肿瘤；头颈区域（例如喉、下咽、鼻咽、口咽、唇、口腔、舌和食道）的肿瘤；和/ 或神经胶质瘤。

在本发明中，EGFR过表达是指与正常上皮细胞表面的EGFR表达水平相比，EGFR的表达水平提高；其具体又可以分为高表达、中度表达和低表达，例如DiFi细胞是EGFR高表达细胞株，LoVo细胞是EGFR中度表达的细胞株，而HT-29细胞是EGFR低表达细胞株 （Wild, R., et al., Mol. Cancer Rher 2006:5(1), p104-113, Cetuximab preclinical antitumor activity (monotherapy and combination based) is not predicted by relative total or activated epidermal growth factor receptor tumor expression levels）。

本发明的抗体药物偶联物可以与已知的用于治疗肿瘤的化疗药物联用，所述化疗药物例如为阿霉素(Adriamycin)、环磷酰胺和紫杉烷类[紫杉醇(Taxol)和多西他赛(Taxotere)]、卡培他滨(Xeloda)、吉西他滨(Gemzar)、长春瑞滨(Navelbine)、他莫昔芬、芳香酶抑制剂（瑞宁得、弗隆、阿诺新）、5-FU 加亚叶酸、伊立替康(camptosar)、奥沙利铂、顺铂、卡铂、雌莫司汀、米托蒽醌(Novantrone)、泼尼松、长春新碱(Oncovin)等，或它们的组合。

在本发明中，“治疗”指试图改变所治疗个体或细胞的自然进程的临床干预，可以是为了预防或在临床病理学的进程中进行。治疗的期望效果包括预防疾病的发生或复发、缓解症状、削弱疾病的任何直接或间接病理学后果、

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预防转移、减缓疾病进展的速率、改善或减轻疾病状态、及免除或改善预后。在有些实施方案中，本发明的抗体或抗体药物偶联物用于延迟疾病或病症的发生，或用于减缓疾病或病症的进展。用于评估疾病的成功治疗和改善的上述参数可以容易的通过内科医师所熟悉的常规规程来测量。对于癌症治疗，可通过例如评估疾病进展前时间 (TTP)和/或测定反应率(RR)来测量功效。

在本发明中，“受试者”指脊椎动物。在某些实施方案中，脊椎动物指哺乳动物。哺乳动物包括，但不限于，牲畜(诸如牛)、宠物(诸如猫、犬、和马)、灵长类动物、小鼠和大鼠。在某些实施方案中，哺乳动物指人。

在本发明中，“有效量”指在必需的剂量和时间上有效实现期望的治疗或预防效果的量。本发明的物质/分子的“治疗有效量”可根据诸如个体的疾病状态、年龄、性别和体重及该物质/分子在个体中引发期望应答的能力等因素而变化。治疗有效量还涵盖该物质/分子的治疗有益效果胜过任何有毒或有害后果的量。“预防有效量”指在必需的剂量和时间上有效实现期望的预防效果的量。通常而非必然，由于预防剂量是在疾病发作之前或在疾病的早期用于受试者的，因此预防有效量会低于治疗有效量。在癌症的情况中，药物的治疗有效量可减少癌细胞数；缩小肿瘤体积；抑制(即一定程度的减缓，优选停止)癌细胞浸润到周围器官中；抑制(即一定程度的减缓，优选停止)肿瘤转移；一定程度的抑制肿瘤生长；和/或一定程度的减轻与癌症有关的一种或多种症状。

对于疾病的预防或治疗，本发明的抗体药物偶联物的适宜剂量(在单独地或联合一种或多种其它别的治疗剂诸如化疗剂地使用时)会取决于待治疗疾病的类型、抗体药物偶联物的类型、疾病的严重程度和进程、施用抗体药物偶联物是出于预防还是治疗目的、先前的疗法、患者的临床病史和对抗体药物偶联物的反应性、及主治医师的判断。合适的是，一次性或通过一系列治疗将抗体药物偶联物施用于患者。根据疾病的类型和严重程度，施用于患者的初始候选剂量可以是约 1μg/kg 至 100mg/kg(例如 0.1mg/kg-20mg/kg)抗体药物偶联物，例如或是通过一次或多次分开施药或是通过连续输注。根据上文所述因素，典型日剂量的范围可以是约 1μg/kg 至 100mg/kg 或更多。对于持续数天或更长时间的重复施药，根据状况，通常持续治疗直至疾病症状发生期望的抑制。抗体药物偶联物的例示剂量的范围可以是约 0.05mg/kg 至约 10mg/kg。如此，可以对患者施用一剂或多剂约 0.5mg/kg、2.0mg/kg、4.0mg/kg 或 10mg/kg(或其任意组合)的抗体药物偶联物。此类剂量可间歇施用，例如每周或每三周(例如使得患者接受约 2 剂至约 20 剂，或例如约 6 剂抗体药物

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偶联物)。可施用一剂较高的初始加载剂量，后续一剂或多剂较低剂量。这种疗法的进程易于通过常规技术和测定法来监测。“长期”施用指与短期模式相反，以连续模式施用药剂，从而将初始治疗效果(活性)维持较长一段时间。“间歇”施用指不是无间断连续进行的治疗，而是本质上周期性的。“联合”一种或多种其它治疗剂的施用包括同时(共同)施用和任意次序的序贯施用。

“药学上可接受的载体”在用于本发明时包括药学可接受的载体、赋形剂或稳定剂，它们在所采用的剂量和浓度对暴露于其的细胞或哺乳动物是无毒的。通常，生理学可接受的载体是 pH 缓冲水溶液。生理学可接受载体的例子包括缓冲剂，诸如磷酸盐、柠檬酸盐和其它有机酸；抗氧化剂，包括抗坏血酸；低分子量(少于约 10 个残基)多肽；蛋白质，诸如血清清蛋白、明胶或免疫球蛋白；亲水性聚合物，诸如聚乙烯吡咯烷酮；氨基酸，诸如甘氨酸、谷氨酰胺、天冬酰胺、精氨酸或赖氨酸；单糖、二糖和其它碳水化合物，包括葡萄糖、甘露糖，蔗糖，海藻糖或糊精；螯合剂，诸如 EDTA；糖醇，诸如甘露醇或山梨醇；成盐反荷离子，诸如钠；和/或非离子表面活性剂，诸如 TWEEN™、聚乙二醇(PEG)和 PLURONICS™。

在本发明中，KRAS 基因与 K-RAS 基因具有相同的含义，其为 RAS 基因家族成员之一，编码 K-ras 蛋白，与多种肿瘤的生成、增殖、迁移、扩散以及血管生成有关。其常见的突变位点为 K-RAS 基因 2 号外显子的 12 号密码子和 13 号密码子，3 号外显子的 61 号密码子，其中有 7 个突变热点：G12C、G12R、G12S、G12V、G12D、G12A、G13V/D.这 7 种突变占到了 90%以上。在本发明的一个实施方案中，所述肿瘤为与 EGFR 过表达相关的 KRAS 基因突变的肿瘤。

在本发明中，BRAF（v-raf murine sarcoma viral oncogene homologB1）基因是一种原癌基因，是 RAF 家族成员之一。大约 8％的人类肿瘤发生 BRAF 突变，BRAF 基因突变的绝大部分突变形式为 BRAFV600E 突变，该突变导致下游 MEK／ERK 信号通路持续激活，对肿瘤的生长增殖和侵袭转移至关重要。在本发明的一个实施方案中，所述肿瘤为与 EGFR 过表达相关的 BRAF 基因突变的肿瘤。

在本发明中，20 种常规氨基酸和其缩写遵从常规用法。参见 Immunology-A Synthesis (第 2 版, E.S. Golub 和 D.R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991))，其通过引用合并入本文。

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附图说明

图 1 测定抗体药物偶联物的药物/抗体比例的 HIC-HPLC 图。

图 2 单抗及抗体药物偶联物的体外细胞活性测定，其中○代表 BA03 单克隆抗体，▲代表 MYK-3 抗体药物偶联物。

图 3 MYK-3 对结肠癌细胞 HT-29 的生长抑制活性，其中○代表 BA03 单克隆抗体，▲代表 MYK-3 抗体药物偶联物。

图 4 MYK-3 对脑胶质瘤癌细胞 U87-MG 的生长抑制活性，其中○代表 BA03 单克隆抗体，▲代表 MYK-3 抗体药物偶联物。

图 5 MYK-3 对肺癌细胞 A549 的生长抑制活性，其中○代表 BA03 单克隆抗体，▲代表 MYK-3 抗体药物偶联物。

图 6 MYK-3 对 KRAS 突变体结肠癌细胞 LoVo 的生长抑制活性，其中代表爱必妥单克隆抗体，▲代表 MYK-3 抗体药物偶联物。

图 7 单抗及抗体药物偶联物对小鼠 HT-29 结肠癌移植瘤体积的影响。图中数据用平均值±标准差表示；与缓冲液对照组相比，*表示 $P<0.05$，**表示 $P<0.01$，***表示 $P<0.001$。

图 8 单抗及抗体药物偶联物对小鼠 HT-29 结肠癌移植瘤模型的体重的影响。

图 9 MYK-3、单抗 BA03 和 BA03+等摩尔数 vcMMAE 对结直肠癌细胞 DiFi 的生长抑制活性，其中，□代表 MYK-3 的单抗组分 BA03，△代表单抗组分 BA03 加上同等于 MYK-3 载药摩尔数的 vcMMAE，◆ 代表 MYK-3。

图 10 MYK-3 对裸鼠中 KRAS 突变体结肠癌细胞 LoVo 的移植瘤生长抑制活性。

图 11 BA03-MCC-MMAE、BA03-MC-MMAE 和 MYK-3 的 HIC-HPLC 分析图谱。

图 12 MYK-3 与 BA03-MC-MMAE、BA03-MCC-MMAE 的体外细胞活性比较，其中，□代表 BA03-MC-MMAE，△代表 BA03-MCC-MMAE，◆代表 MYK-3。

具体实施方式

下面将结合实施例对本发明的实施方案进行详细描述，但是本领域技术人员将会理解，下列实施例仅用于说明本发明，而不应视为限定本发明的范围。实施例中未注明具体条件者，按照常规条件或制造商建议的条件进行。所用试剂或仪器未注明生产厂商者，均为可以通过市购获得的常规

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产品。

本发明中的抗体 BA03 即中国发明专利申请 CN 103772504A 中的 BA03，其制备方法参考该专利申请中的实施例 3，该抗体各部分的序列如下：

重链可变区序列为：

QVQLQESGPGLVKPSETLSLTCTVSGFSLSNYDVHWVRQAPGKGLEWLGVIWSGGNTDYNTPFTSRLTISVDTSKNQFSLKLSSVTAADTAVYYCARALDYYDYEFAYWGQGTLVTVSS（SEQ ID NO: 1）。

其中下划线部分分别为 CDR1（SEQ ID NO: 5）、CDR2（SEQ ID NO: 6）、CDR3（SEQ ID NO: 7）；

未下划线部分分别为 FR1（SEQ ID NO: 8）、FR2（SEQ ID NO: 9）、FR3（SEQ ID NO: 10）、FR4（SEQ ID NO: 11）。

轻链可变区序列为：

EIVLTQSPDFQSVTPKEKVTITCRASQSIGTNIHWYQQKPDQSPKLLIKYASESISGIPSRFSGSGSGTDFTLTINSLEAEDAATYYCQQNNEWPTSFGQGTKLEIK（SEQ ID NO: 2）。

其中下划线部分分别为 CDR1（SEQ ID NO: 12）、CDR2（SEQ ID NO: 13）、CDR3（SEQ ID NO: 14）；

未下划线部分分别为 FR1（SEQ ID NO: 15）、FR2（SEQ ID NO: 16）、FR3（SEQ ID NO: 17）、FR4（SEQ ID NO: 18）。。

重链恒定区序列为：

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK（SEQ ID NO: 3）。

轻链恒定区序列为：

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC（SEQ ID NO: 4）。

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实施例 1 抗体药物偶联物的制备方法

取 10 毫克的 BA03 抗体，使用 15mL 的 30KD 超滤装置置换到还原缓冲液 (25mM 硼酸钠, pH8.0, 25mM NaCl, 5 mM EDTA)中，共置换三次；终体积约为 1mL,转移至新的 Eppendorf 离心管（称重）中，并称重;检测蛋白浓度，计算蛋白总量。向抗体中加入 2.5 倍摩尔数的 DTT，室温保温 2 小时，连续混匀；使用 15ml 的 30KD 超滤装置置换到偶联缓冲液 （50mM Tris, pH7.2, 150mM NaCl, 5 mM EDTA）中，共置换三次。取浓缩液，以 A280 测定蛋白浓度，并称重，计算蛋白总量；取 10μl 样品，以 Ellman's 方法测定自由巯基数;

并以下列公式计算其自由巯基的摩尔浓度:

$$C_{mol}=\frac{A412\times 11.2}{b\times 14150}\ (M)$$

b: 比色皿光路长度（通常 1cm）。

根据自由巯基的摩尔浓度和总蛋白溶液体积计算自由巯基摩尔数。

向还原后的抗体中加入 1.1 倍于自由巯基摩尔数的 vc-MMAE（购上海皓元化学科技有限公司，货号 HY-15575）（溶于 DMSO），混匀后室温反应 2 小时，间断混匀。向反应体系中加入 20 倍于所投入之 vc-MMAE 摩尔数的 N-乙酰半胱氨酸于反应液中，混匀，静置 5 分钟。使用 15ml 的 30KD 超滤装置置换到偶联物储存液（20mM 柠檬酸钠(Na-citrate), 0.3% NaCl, 5% 海藻糖（Trehalose）, 0.05% TWeen-80, pH6.0）中，共置换三次，即得到抗体药物偶联物 MYK-3；样品于 4℃保存。

药物/抗体比例的测定:

将制备好的抗体药物偶联物 MYK-3 经过 HIC-HPLC 分析（Jun Ouyang, Drug-To-Antibody(DAR) Ratio and Drug Distributionby Hydrophobic Interaction Chromatography and Reverse Phase High Performance Chromatography, Laurent Ducry(ed.), Antibody Drug Conjugates, Chapter 17, Methods in Molecular Biology, Vol 1045, p275-283）以测定药物/抗体比例（drug antibody ratio， DAR），见图 1，根据图谱峰面积计算得到平均载药数 DAR 为 4.1。

实施例 2 抗体药物偶联物 MYK-3 体外细胞活性的测定

细胞活性检测方法:

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1.1 复苏后的细胞株经过3-4代细胞传代之后，先倒去培养基，用5mL DPBS润洗一遍，再用3mL胰蛋白酶消化细胞，分别用培养基重悬，离心机离心，弃上清，再用培养基再次重悬，取出0.5mL用细胞计数仪计数。在96孔细胞培养板上进行铺板（DiFi细胞以 10000个细胞/孔，HT-29细胞以 5000个细胞/孔, A549细胞以2000个细胞/孔，U87-MG细胞以 3000个细胞/孔， LoVo细胞以4000个细胞/孔）， 培养24小时后，加入系列稀释浓度的单克隆抗体BA03以及抗体药物偶联物MYK-3，保温72小时，之后每孔加入20μl CCK8显色试剂，在波长450-650nm用酶标仪检测OD450-650，并进行四参数拟合。

体外细胞活性实验结果：

以下各细胞株购自中国科学院上海细胞生物学研究所。

在EGFR 高表达的DiFi细胞（人结直肠癌细胞）中的活性： MYK-3比单抗BA03有显著增高的细胞生长抑制活性， EC50 降低了大约10倍（BA03的EC50是51.9 ng/ml， MYK-3的EC50是 5.1 ng/ml)， 如图2所示。

在EGFR中度表达和低度表达的其它肿瘤细胞中的活性： MYK-3相对于单抗本身对 EGFR 中度表达和低度表达的癌细胞（人结肠癌细胞HT29、人肺癌细胞A549、人脑星形胶质母细胞瘤细胞U87-MG）也显示了明显的细胞生长抑制活性（如图3、图4、图5所示），其中HT-29 的 EC50是611 ng/ml, A549 的EC50是28.3 μg/ml，U87-MG 的EC50是5.3 μg/ml。

另外，我们还测试了在 EGFR 中度表达的 KRAS 突变体结肠癌细胞LoVo (Dunn EF, Ilda M, Myers RA, Hintz KA, Campbell DA, Armstrong EA, Li C and Wheeler DL. Dasatinib sensitizes KRAS mutant colorectal tumors to cetruximab. Oncogene 2011; 30:561-574)中的活性，发现MYK-3对KRAS 突变体结肠癌细胞LoVo显示了明显的肿瘤生长抑制活性(如图6所示，EC50 是 3.2μg/ml)，而 BA03 单独使用时对该细胞株几乎没有抑制活性。

实施例3 小鼠体内移植瘤实验

小鼠体内移植瘤实验方法：

HT-29结肠癌细胞是EGFR低表达并带有BRAF突变的细胞株，目前已在市场上销售的治疗结直肠癌的 EGFR 靶向单抗爱必妥(Erbitux) 对 HT-29细胞株没有生长抑制活性。

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HT-29 细胞移植模型：收集对数生长期的肿瘤细胞，计数后重悬于1×PBS，调整细胞悬液浓度至 3×10^7/ml。用 1ml 注射器（4 号针头）在裸鼠右侧背部皮下接种肿瘤细胞，3×10^6/0.1ml/鼠，当肿瘤体积达到 150-200mm^3 后用随机区组法分组，每组 8 只小鼠，保证各组间肿瘤体积和小鼠体重均一。各组肿瘤体积的均值与所有实验动物肿瘤体积的均值差异不超过±10％。 尾静脉给药，每四天给一次药（第 1、5、9、13 天），一共给药 4 次，定期测量肿瘤体积和小鼠体重。每一个给药组分别有 8 只小鼠。

小鼠移植瘤实验结果

小鼠 HT-29 结肠癌移植瘤实验：实验共分五组， 包括缓冲液组（20 mM 柠檬酸钠，0.3%氯化钠，5%海藻糖，0.05%吐温 80， pH6）， BA03 单抗组（5mg/kg）、MYK-3 组（1mg/kg）、MYK-3 组（5mg/kg）和非结合 ADC 组（5mg/kg）（人源 IgG-vcMMAE 偶联物，其中 IgG 是从健康人血清纯化的 IgG，该偶联物制备方法与 MYK-3 相同）。MYK-3 给药的小鼠移植肿瘤体积与对照组比较显著降低，显示了明显的抗肿瘤生长效应（图 7）。在第 18 天，MYK-3 在 5mg/Kg 的给药剂量组与缓冲液组相比肿瘤生长抑制率达到 54%，与单抗 BA03 的同等剂量组相比肿瘤生长抑制率达到 46%， 与非结合 ADC 相比肿瘤生长抑制率达到 42%.

小鼠体重：MYK-3 给药的小鼠体重与对照组比较没有变化（见图 8），说明 MYK-3 没有使小鼠体重降低的毒性作用。

实施例 4 MYK-3、单抗 BA03 和 BA03+等摩尔数 vcMMAE 对结直肠癌细胞 DiFi 的生长抑制活性

按照实施例 2 方法检测了 MYK-3、单抗 BA03 和 BA03+等摩尔数 vcMMAE 对结直肠癌细胞 DiFi 的生长抑制活性，实验结果见图 9，其中的 EC50 值分别为 8.4 ng/mL, 65.8 ng/mL 和 68.2 ng/mL。

从图中可以看出，单抗 BA03 在 EGFR 高表达的结直肠癌细胞 DiFi 中显示了一定的生长抑制活性；并且 BA03 加上同等于 MYK-3 载药摩尔数的游离 vcMMAE 与单独的 BA03 活性比较没有明显区别；然而，BA03 与 vcMMAE 偶联形成的 MYK-3 作为 ADC 分子对 DiFi 结直肠癌细胞的生长抑制活性大大高于单抗 BA03 本身以及 BA03 加上同等于 MYK-3 载药摩尔数的游离 vcMMAE 的活性，EC50 提高了大约 8 倍。

实施例 5 MYK-3 对裸鼠中 KRAS 突变体结肠癌细胞 LoVo 的移植瘤生

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长抑制活性

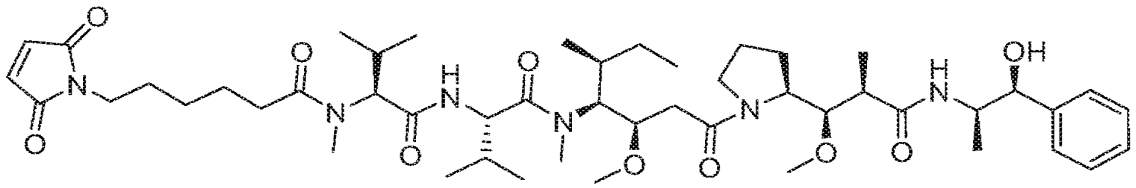
按照实施例 3 的模型构建（以 2×10^6/0.1ml/鼠接种肿瘤细胞）和给药方法检测 MYK-3 对裸鼠中 KRAS 突变体结肠癌细胞 LoVo 的移植瘤生长抑制活性，实验共分六组，包括稀释缓冲液组（20 mM 柠檬酸钠，0.3%氯化钠，5%海藻糖，0.05%吐温 80， pH6），爱必妥单抗组（3mg/kg）、MYK-3 组（0.3mg/kg、1mg/kg、3mg/kg 三个剂量）和非结合对照 ADC 组（3mg/kg），其中非结合对照 ADC 代表非结合的同等载药量的 ADC 对照（non-binding ADC control,为 anti-CD20 mAb-vcMMAE），该偶联物制备方法与 MYK-3 相同。实验结果见图 10。

从图中可以看出，MYK-3 在 3 mg/Kg 剂量下表现出对 LoVo 细胞肿瘤生长的完全抑制，并且 MYK-3 在 1 mg/Kg 已显示出比上市药品 Erbitux 在 3 mg/Kg 剂量下还更强的活性。

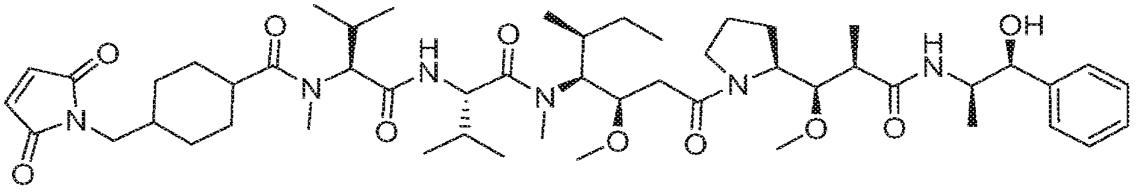
实施例 6 MYK-3 与 BA03-MC-MMAE、 BA03-MCC-MMAE 的体外细胞活性比较

MC-MMAE 和 MCC-MMAE 的结构如下所示:

MC-MMAE:



MCC-MMAE:



MC-MMAE 和 MCC-MMAE 的制备方法参见实施例 1。

药物/抗体比例的测定： 使用 HIC-HPLC 分析方法测定药物/抗体比例，具体参见实施例 1，测得的 MYK-3（即为 BA03-vcMMAE），BA03-MC-MMAE

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和 BA03-MCC-MMAE 的药物/抗体比例均为 3.9，参见图 11。

体外细胞活性实验方法参见实施例 2；简而言之，取一定数量的 DiFi 细胞株，接种于 96 孔板中，培养 24 小时后加入不同浓度梯度稀释的 BA03-MC-MMAE，BA03-MCC-MMAE，MYK-3 三个样品。再培养 96 小时，加入 CCK-8 检测试剂显色，在酶标仪上读取 96 孔板每个孔的 OD 值，检测不同浓度样品的活细胞数量，判断样品对 DiFi 细胞株的增殖抑制效果。实验结果见图 12，其中 BA03-MC-MMAE、BA03-MCC-MMAE 和 MYK-3 的 EC50 值分别为 72.6 ng/mL，71.6 ng/mL 和 9.8 ng/mL。

由图中可见，MYK-3 的细胞增殖抑制活性明显高于 BA03-MC-MMAE 和 BA03-MCC-MMAE 的活性；事实上，BA03-MC-MMAE 和 BA03-MCC-MMAE 的细胞增殖抑制活性与单抗 BA03 本身的活性相似。这说明使用同样的单抗和细胞毒小分子，但使用不同的链接体可以造成 ADC 的活性有显著差异。

尽管本发明的具体实施方式已经得到详细的描述，本领域技术人员将会理解。根据已经公开的所有教导，可以对那些细节进行各种修改和替换，这些改变均在本发明的保护范围之内。本发明的全部范围由所附权利要求及其任何等同物给出。

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权 利 要 求 书

1. 抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其包含与细胞毒剂共价连接的抗表皮生长因子受体抗体。

2. 权利要求 1 的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的抗表皮生长因子受体抗体包括重链和轻链，其中重链可变区 CDR1、CDR2、CDR3 分别包含如 SEQ ID NO: 5～7 所示的序列或其突变体，轻链可变区 CDR1、CDR2、CDR3 分别包含如 SEQ ID NO: 12～14 所示的序列或其突变体。

3. 权利要求 2 的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的抗表皮生长因子受体抗体重链可变区 FR1、FR2、FR3、FR4 区分别包含如 SEQ ID NO: 8～11 所示的序列或其突变体。

4. 权利要求 2 的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的抗表皮生长因子受体抗体轻链可变区 FR1、FR2、FR3、FR4 区分别包含如 SEQ ID NO: 15～18 所示的序列或其突变体。

5. 权利要求 1-4 任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的抗表皮生长因子受体抗体的重链恒定区选自人源性 IgG、IgM、IgA、IgD、IgA 恒定区或上述恒定区的突变体。

6. 权利要求 5 的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述 IgG 选自 IgG1、IgG2、IgG3 和 IgG4。

7. 权利要求 5 的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的抗表皮生长因子受体抗体重链恒定区的氨基酸序列包含如 SEQ ID NO: 3 所示的序列，或者包含与 SEQ ID NO: 3 所示序列的同一性大于 70%，例如大于 75%、80%、85%、90%、95%、

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99%的序列。

8. 权利要求 1-7 任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的抗表皮生长因子受体抗体的轻链恒定区为人源性 lambda 恒定区、kappa 恒定区、或上述恒定区的突变体。

9. 权利要求 8 的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的抗表皮生长因子受体抗体轻链恒定区的氨基酸序列包含如 SEQ ID NO: 4 所示的序列，或者包含与 SEQ ID NO: 4 所示序列的同一性大于 70%，例如大于 75%、80%、85%、90%、95%、99%的序列。

10. 权利要求 1-9 任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其具有式 I 所示的结构，

$$Ab-(L-D)_p$$

式 I

其中：

Ab 代表抗表皮生长因子受体抗体；

L 代表接头；

D 代表细胞毒剂；

p 代表 1-9，例如 2-6，例如 3-5。

11. 权利要求1-10任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的细胞毒剂选自化疗药物、毒素、放射性同位素、细胞因子、抗生素、酶、纳米颗粒和生物活性肽。

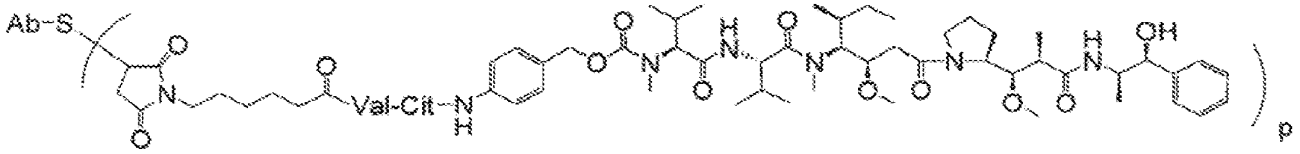
12. 权利要求11的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的细胞毒剂选自Monomethyl auristatin E（MMAE）、 Monomethyl auristatin F（MMAF）、 美登木素生物碱（例如Maytansine DM1、Maytansine DM4）、卡奇霉素(calicheamicin)、duocarmycin MGBA、阿霉素(doxorubicin)、蓖麻毒素、白喉毒素等毒素、I131、白介素类、肿瘤坏死因子、趋化因子和纳米颗粒等，优选为MMAE。

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13. 权利要求 10 的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的接头为可切割的或不可切割的。

14. 权利要求 13 的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的接头选自 6-马来酰亚氨基己酰基(MC)、马来酰亚氨基丙酰基(MP)、缬氨酸-瓜氨酸(val-cit)、丙氨酸-苯丙氨酸(ala-phe)、对氨基苄氧羰基(PAB)、N-琥珀酰亚氨基 4-(2-吡啶基硫代)戊酸酯(SPP)、N-琥珀酰亚氨基 4-(N-马来酰亚氨基甲基)-环己烷-1-羧酸酯(SMCC)、N-琥珀酰亚氨基(4-碘-乙酰基)氨基苯甲酸酯(SIAB)、和 6-马来酰亚氨基己酰基-缬氨酸-瓜氨酸-对氨基苄氧羰基(MC-vc-PAB)，优选为 6-马来酰亚氨基己酰基-缬氨酸-瓜氨酸-对氨基苄氧羰基(MC-vc-PAB)。

15. 权利要求 1-14 任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其为：



其中Ab为抗表皮生长因子受体抗体，p为1-8，例如2-6，例如3-5。

16. 组合物，其含有权利要求1-15任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，任选地，还含有至少一种药学上可接受的载体、稀释剂或赋形剂。

17. 权利要求1-15任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物在制备预防和/或治疗与表皮生长因子受体（EGFR）相关的疾病的药物中的用途。

18. 权利要求16的用途，其中所述的与表皮生长因子受体（EGFR）相关的疾病为与EGFR相关的肿瘤，例如与EGFR过表达相关的肿瘤，例如选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

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19. 权利要求1-15任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物在制备抑制肿瘤血管生成、延缓肿瘤进展、抑制肿瘤生长、抑制肿瘤细胞增殖的药物中的用途。

20. 权利要求19的用途，其中所述的肿瘤选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

21. 权利要求18或20的用途，其中所述的肿瘤为KRAS基因突变的肿瘤，例如为KRAS基因突变的结肠癌、直肠癌、肺癌或胰腺癌。

22. 权利要求18或20的用途，其中所述的肿瘤为BRAF基因突变的肿瘤，例如选自BRAF基因突变的结肠癌、直肠癌和肺癌。

23. 预防和/或治疗与表皮生长因子受体（EGFR）相关的疾病的方法，所述方法包括给予有需要的受试者预防和/或治疗有效量的权利要求1-15任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物。

24. 权利要求23的方法，其中所述的与表皮生长因子受体（EGFR）相关的疾病为与EGFR相关的肿瘤，例如与EGFR过表达相关的肿瘤，例如选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

25. 抑制肿瘤血管生成、延缓肿瘤进展、抑制肿瘤生长、抑制肿瘤细胞增殖的方法，所述方法包括给予有需要的受试者预防和/或治疗有效量的权利要求1-15任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物。

26. 权利要求25的方法，其中所述的肿瘤选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

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27. 权利要求24或26的方法，其中所述的肿瘤为KRAS基因突变的肿瘤，例如为KRAS基因突变的结肠癌、直肠癌、肺癌或胰腺癌。

28. 权利要求24或26的方法，其中所述的肿瘤为BRAF基因突变的肿瘤，例如选自BRAF基因突变的结肠癌、直肠癌和肺癌。

29. 权利要求1-15任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其用于预防和/或治疗与表皮生长因子受体（EGFR）相关的疾病。

30. 权利要求29的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的与表皮生长因子受体（EGFR）相关的疾病为与EGFR相关的肿瘤，例如与EGFR过表达相关的肿瘤，例如选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

31. 权利要求1-15任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其用于抑制肿瘤血管生成、延缓肿瘤进展、抑制肿瘤生长、抑制肿瘤细胞增殖。

32. 权利要求31的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的肿瘤选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

33. 权利要求30或32的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的肿瘤为KRAS基因突变的肿瘤，例如为KRAS基因突变的结肠癌、直肠癌、肺癌或胰腺癌。

34. 权利要求30或32的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的肿瘤为BRAF基因突变的肿瘤，例如选自BRAF基因突变的结肠癌、直肠癌和肺癌。

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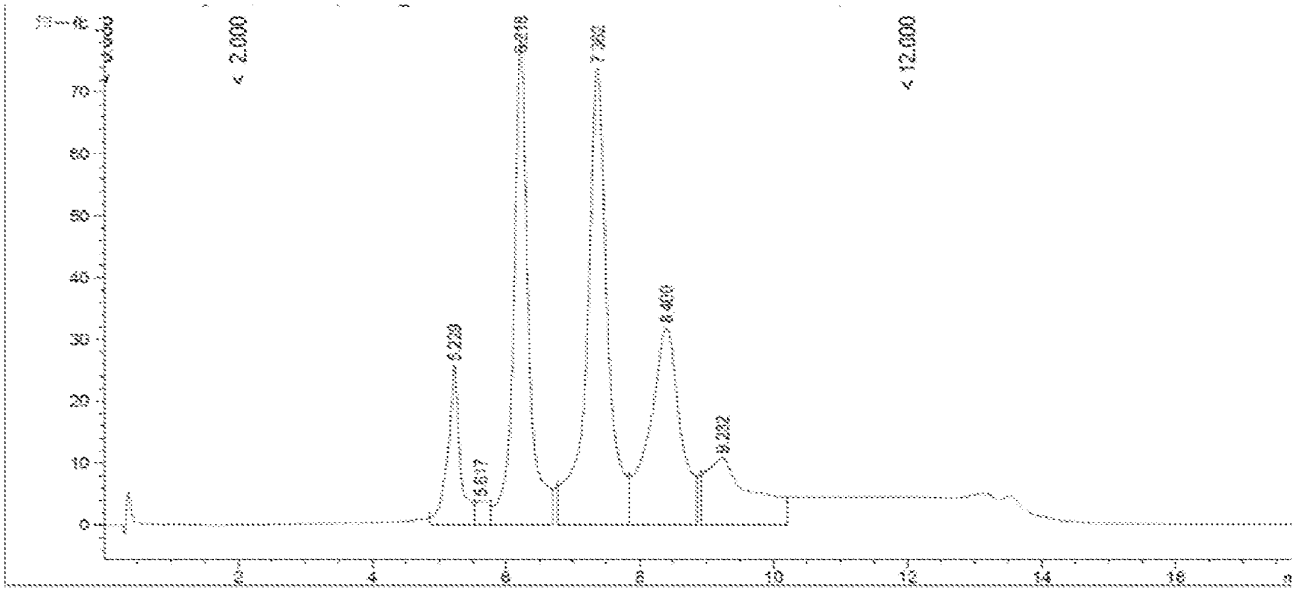


图1

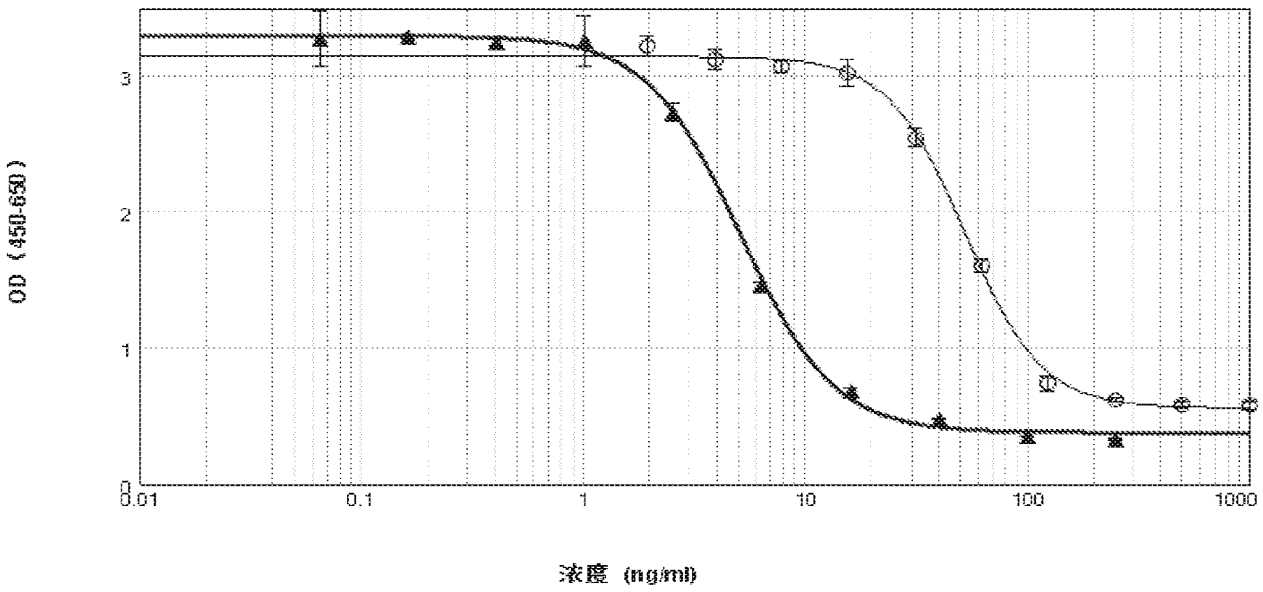


图2

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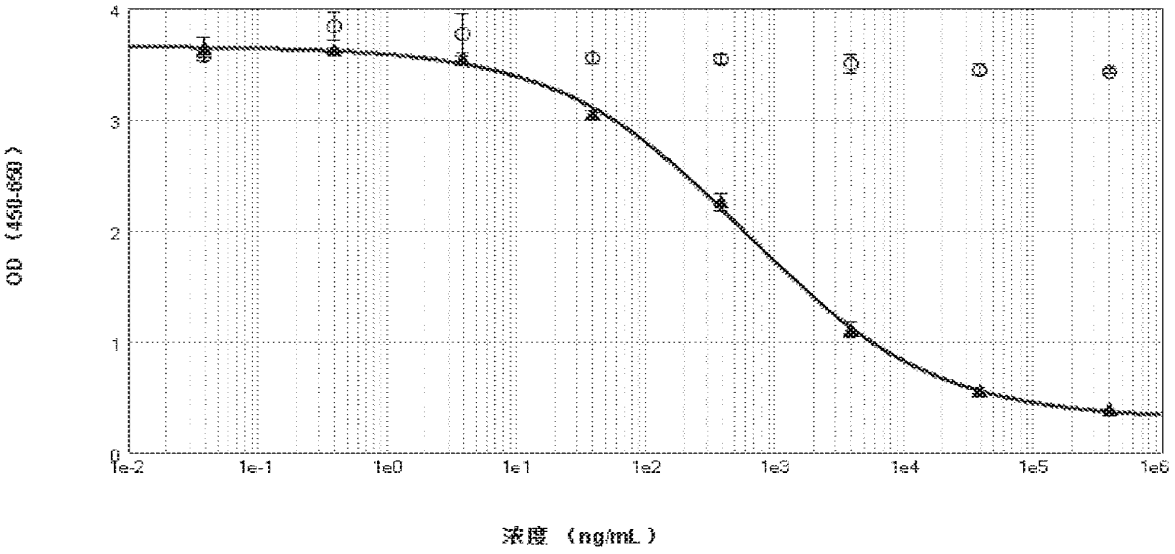

OD（450-650）
4
3
2
1
0
1e-2
1e-1
1e0
1e1
1e2
1e3
1e4
1e5
1e6
浓度（ng/mL）

图3

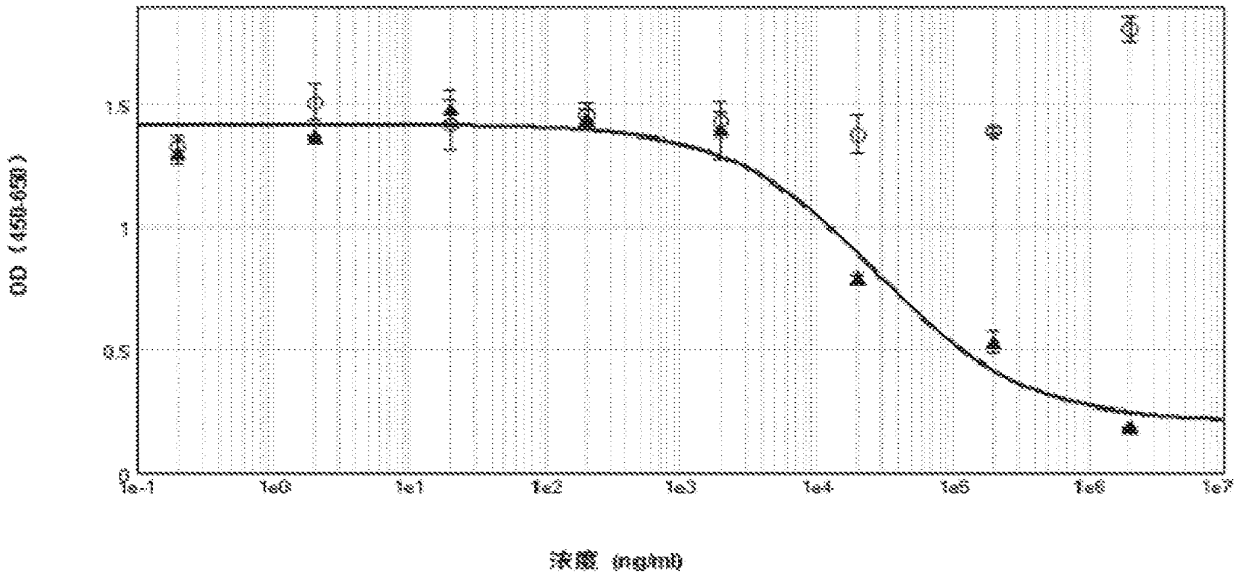

OD（450-650）
1.5
1
0.5
0
1e-1
1e0
1e1
1e2
1e3
1e4
1e5
1e6
1e7
浓度 (ng/ml)

图4

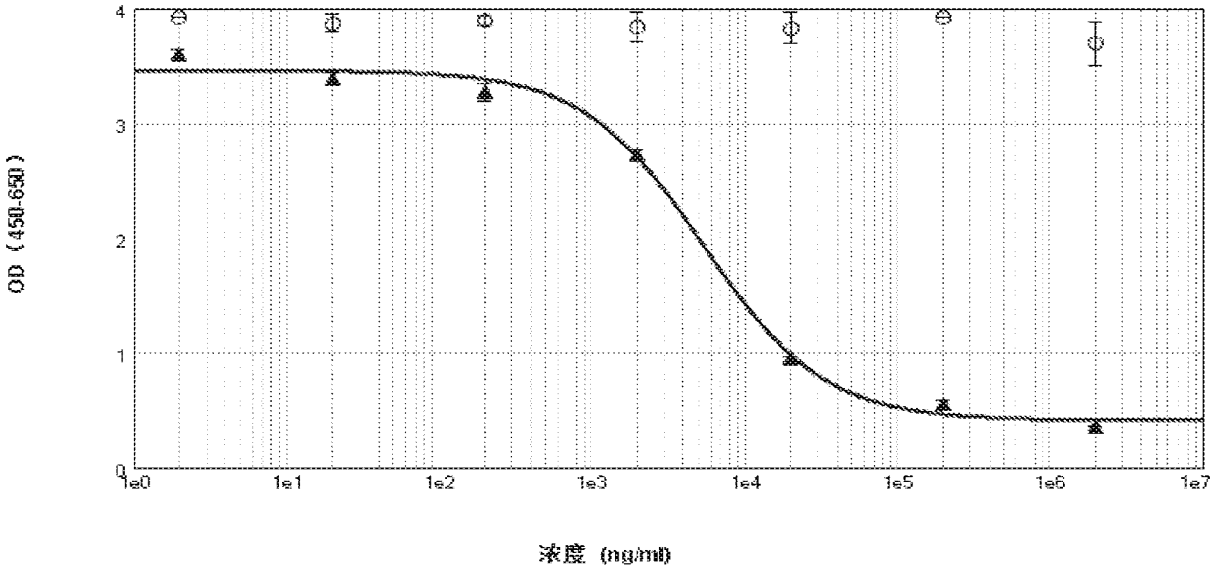

OD（450-650）
4
3
2
1
0
1e0
1e1
1e2
1e3
1e4
1e5
1e6
1e7
浓度（ng/ml）

图5

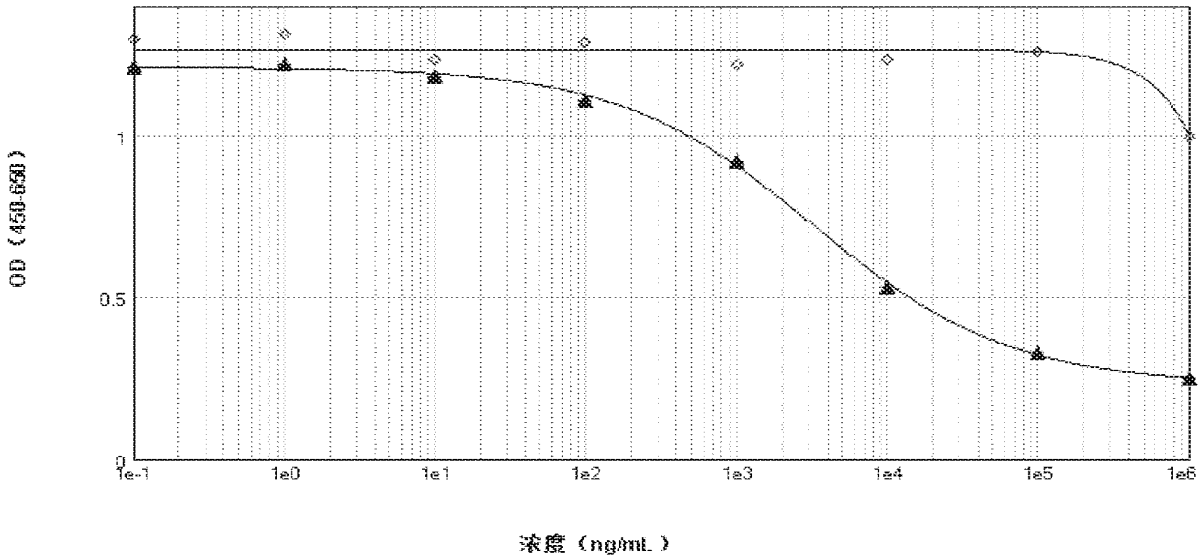

OD（450-650）
1
0.5
0
1e-1
1e0
1e1
1e2
1e3
1e4
1e5
1e6
浓度（ng/mL）

图6

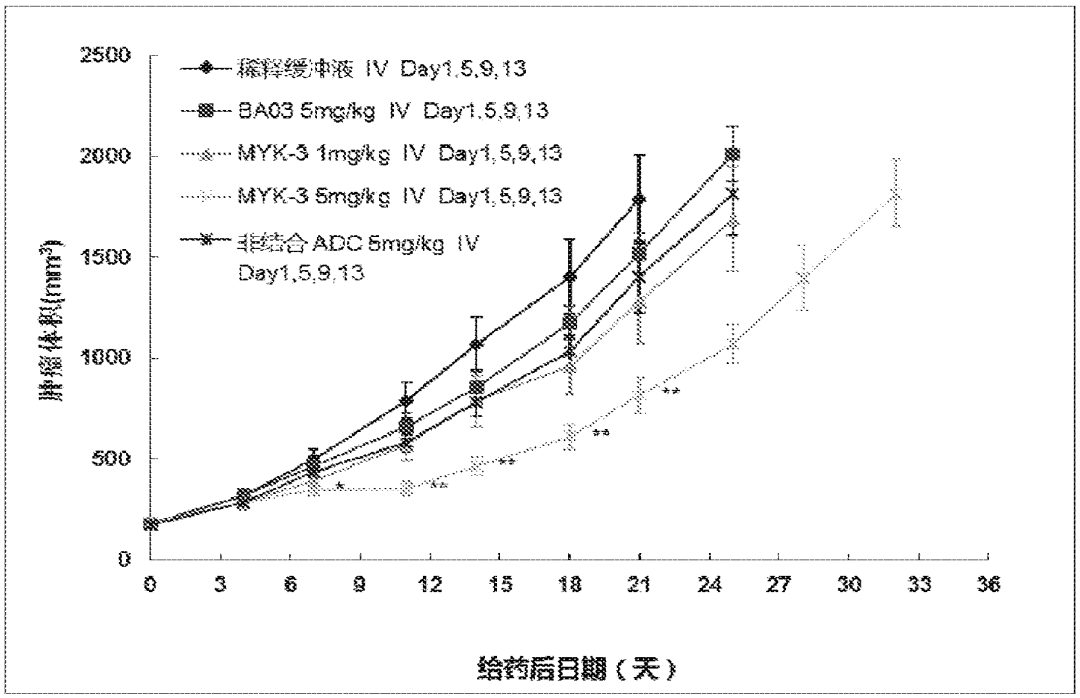


图 7

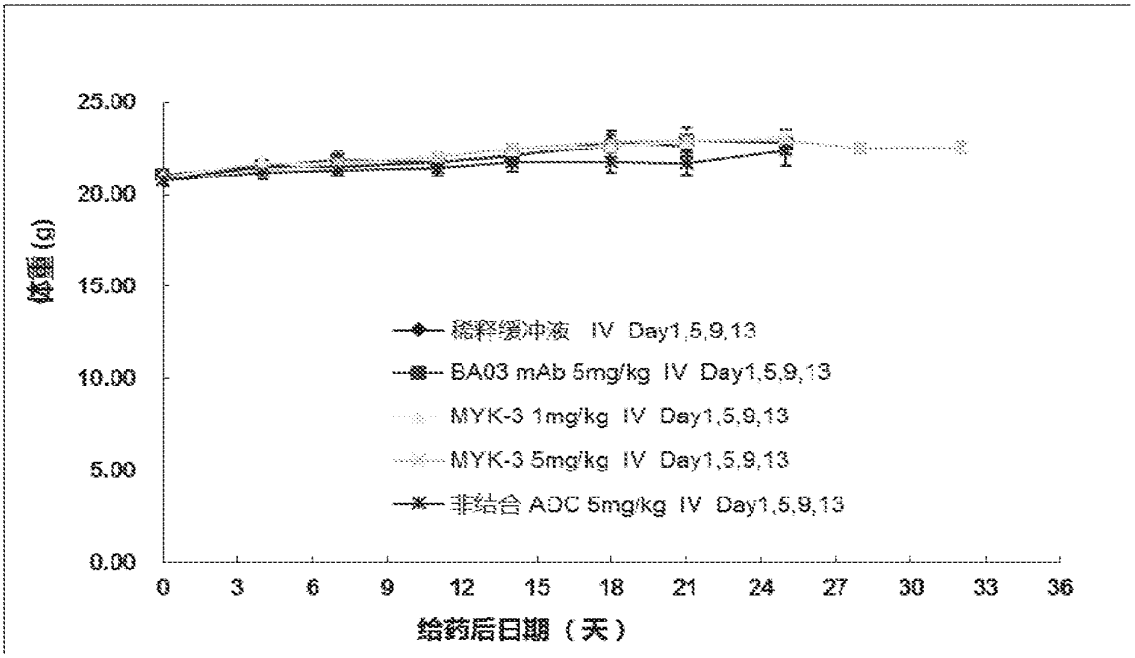


图 8

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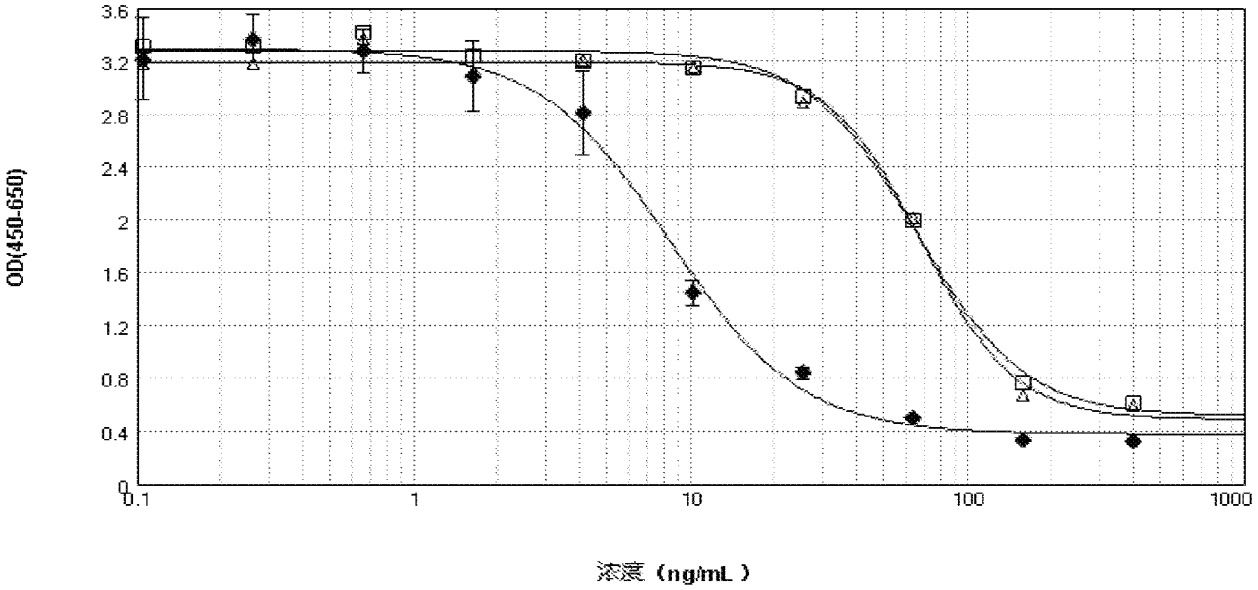


图9

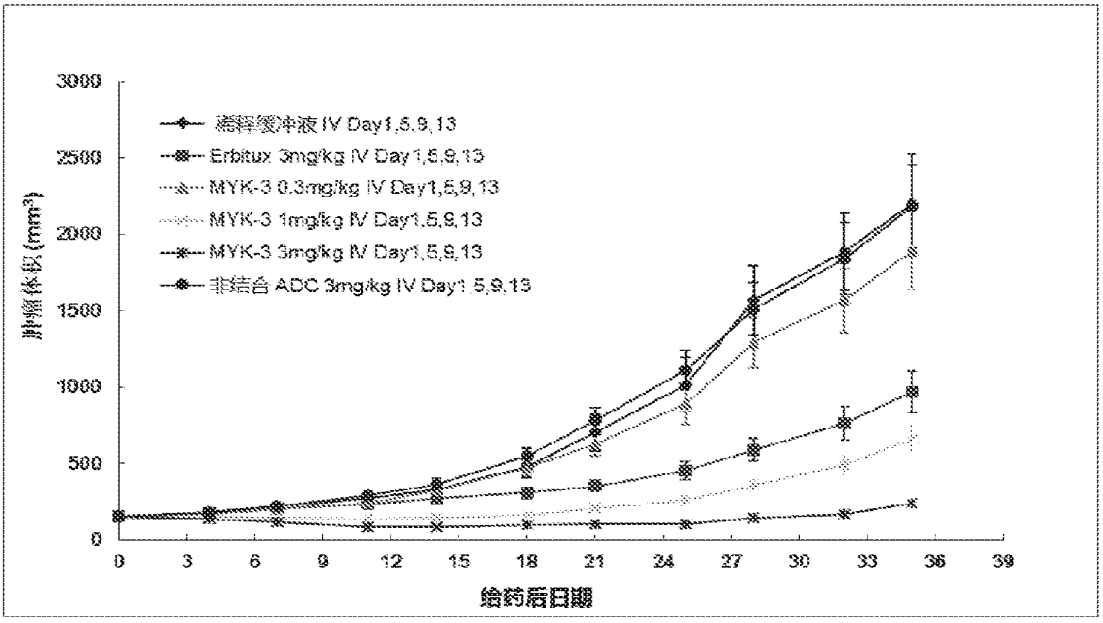


图10

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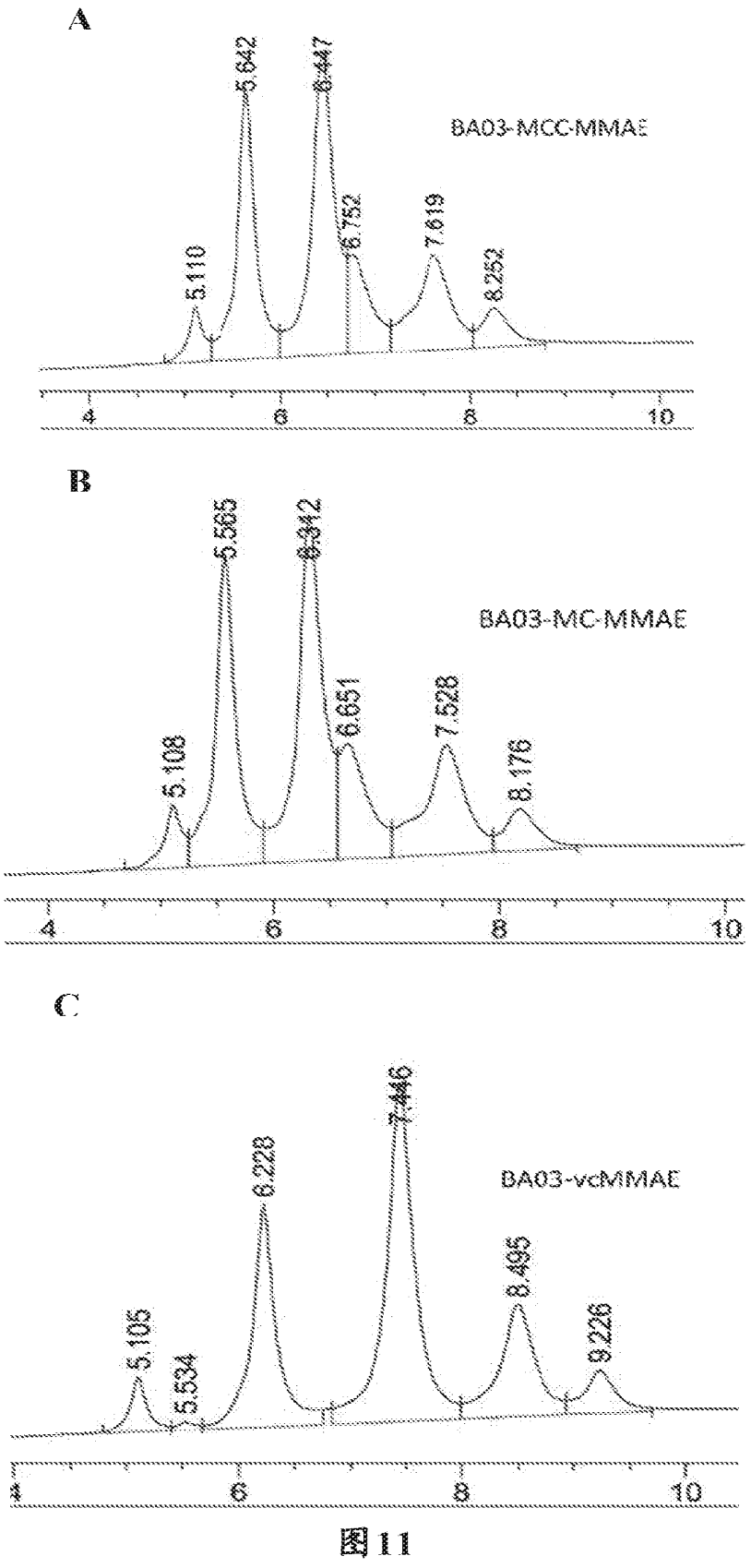

A
5.642
8.447
BA03-MCC-MMAE
6.752
7.619
5.110
8.252
4
6
8
10
B
5.565
6.342
BA03-MC-MMAE
6.651
7.528
5.108
8.176
4
6
8
10
C
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6.228
BA03-vcMMAE
8.495
5.105
9.226
5.534
4
6
8
10

图 11

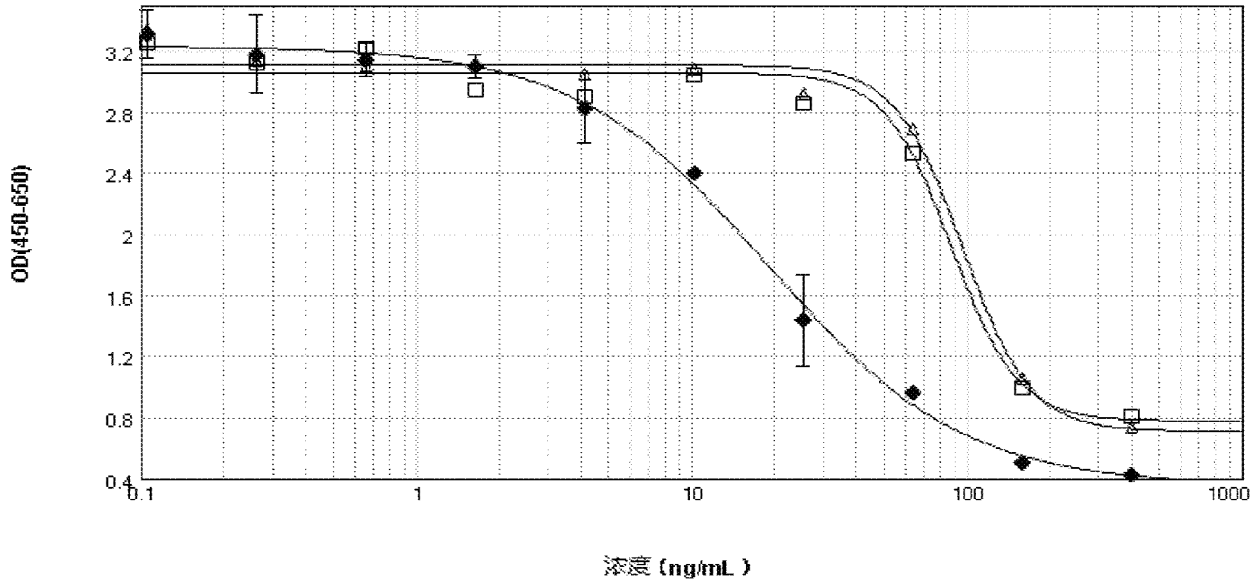


图 12

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2016/073844

A. CLASSIFICATION OF SUBJECT MATTER

A61K 47/48 (2006.01) i; A61K 45/00 (2006.01) i; A61P 35/00 (2006.01) i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K, A61P

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)

CPRSABS; CNKI; CNTXT and Search Terms: epidermal growth factor receptor, antibody, cytotoxic drug, antibody-drug-conjugate, valine, citrulline etc.

DWPI; SIPOABS; EPTXT; USTXT; WOTXT; JPTXT; ELSEVIER; CA and Search Terms: epidermal growth factor receptor, EGFR, HER, antibody, cytotoxic drug, MMAE, antibody-drug-conjugate, ADC, valine, citrulline etc.

NATIONAL BIO-SEQUENCE DATABASE OF CHINESE PATENT; Genbank; EMBL; STN and Sequences Searched: SEQ ID NOs: 1-18

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014152199 A1 (ABBVIE INC.), 25 September 2014 (25.09.2014), see abstract, claims 1-50, description, page 25, line 1 to page 36, line 3, and figure 1	1, 5-22 and 29-34
Y	WO 2014152199 A1 (ABBVIE INC.), 25 September 2014 (25.09.2014), see abstract, claims 1-50, description, page 25, line 1 to page 36, line 3, and figure 1	2-22 and 29-34
Y	CN 103772504 A (SHANGHAI JMT BIOTECHNOLOGY CO., LTD.), 07 May 2014 (07.05.2014), see claims 1-13, and embodiments 1-10	2-22 and 29-34

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 May 2016 (12.05.2016)	**18 May 2016 (18.05.2016)**
Name and mailing address of the ISA/CN: State Intellectual Property Office of the P. R. China No. 6, Xitucheng Road, Jimenqiao Haidian District, Beijing 100088, China Facsimile No.: (86-10) 62019451	Authorized officer **HE, Rui** Telephone No.: (86-10) **62411106**

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2016/073844

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 23-28
because they relate to subject matter not required to be searched by this Authority, namely:
[1] PCT Rule 39.1(iv) - a surgical method or a treatment method for the disposal of a human body or animal body.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2016/073844

Patent Documents referred in the Report	Publication Date	Patent Family	Publication Date
WO 2014152199 A1	25 September 2014	KR 20150132864 A	26 November 2015
		AU 2014240012 A1	24 September 2015
		IL 241004 D0	30 November 2015
		SG 11201507432X A	29 October 2015
		CN 105209076 A	30 December 2015
		EP 2968589 A1	20 January 2016
		CA 2906022 A1	25 September 2014
		US 2014286968 A1	25 September 2014
		TW 201519904 A	01 June 2015
CN 103772504 A	07 May 2014	None	

国际检索报告

国际申请号
PCT/CN2016/073844

A. 主题的分类

A61K 47/48(2006.01)i；A61K 45/00(2006.01)i；A61P 35/00(2006.01)i

按照国际专利分类(IPC)或者同时按照国家分类和IPC两种分类

B. 检索领域

检索的最低限度文献(标明分类系统和分类号)

A61K，A61P

包含在检索领域中的除最低限度文献以外的检索文献

在国际检索时查阅的电子数据库(数据库的名称，和使用的检索词（如使用））

CPRSABS；CNKI；CNTXT和检索词：表皮生长因子受体，抗体，细胞毒剂药物，抗体药物缀合物，缬氨酸，瓜氨酸等 DWPI；SIPOABS；EPTXT；USTXT；WOTXT；JPTXT；ELSEVIER；CA和检索词：epidermal growth factor receptor，EGFR，HER，antibody，cytotoxic drug，MMAE，antibody-drug-conjugate，ADC，valine，citrulline等 中国专利生物序列检索系统；Genbank；EMBL；STN和检索的序列：SEQ ID NOs：1-18

C. 相关文件

类 型*	引用文件，必要时，指明相关段落	相关的权利要求
X	WO 2014152199 A1 (ABBVIE INC) 2014年 9月 25日 (2014-09-25) 参见摘要，权利要求1-50，说明书第25页第1行至第36页第3行，图1	1、5-22和29-34
Y	WO 2014152199 A1 (ABBVIE INC) 2014年 9月 25日 (2014-09-25) 参见摘要，权利要求1-50，说明书第25页第1行至第36页第3行，图1	2-22和29-34
Y	CN 103772504 A (上海津曼特生物科技有限公司) 2014年 5月 7日 (2014-05-07) 参见权利要求1-13，实施例1-10	2-22和29-34

☐ 其余文件在C栏的续页中列出。

☑ 见同族专利附件。

* 引用文件的具体类型：

“A” 认为不特别相关的表示了现有技术一般状态的文件

“E” 在国际申请日的当天或之后公布的在先申请或专利

“L” 可能对优先权要求构成怀疑的文件，或为确定另一篇引用文件的公布日而引用的或者因其他特殊理由而引用的文件（如具体说明的）

“O” 涉及口头公开、使用、展览或其他方式公开的文件

“P” 公布日先于国际申请日但迟于所要求的优先权日的文件

“T” 在申请日或优先权日之后公布，与申请不相抵触，但为了理解发明之理论或原理的在后文件

“X” 特别相关的文件，单独考虑该文件，认定要求保护的发明不是新颖的或不具有创造性

“Y” 特别相关的文件，当该文件与另一篇或者多篇该类文件结合并且这种结合对于本领域技术人员为显而易见时，要求保护的发明不具有创造性

“&” 同族专利的文件

国际检索实际完成的日期	国际检索报告邮寄日期
2016年 5月 12日	2016年 5月 18日
ISA/CN的名称和邮寄地址 中华人民共和国国家知识产权局(ISA/CN) 中国北京市海淀区蓟门桥西土城路6号 100088 传真号 (86-10)62019451	受权官员 何睿 电话号码 (86-10)62411106

表 PCT/ISA/210 (第2页) (2009年7月)

第II栏　　某些权利要求被认为是不能检索的意见(续第1页第2项)

根据条约第17条(2)(a)，对某些权利要求未做国际检索报告的理由如下:

1. ☑ 权利要求： 23-28
因为它们涉及不要求本单位进行检索的主题，即：

[1] PCT细则39.1（iv）——处置人体或者动物体的外科手术方法或治疗方法，以及诊断方法。

2. ☐ 权利要求：
因为它们涉及国际申请中不符合规定的要求的部分，以致不能进行任何有意义的国际检索， 具体地说：

3. ☐ 权利要求：
因为它们是从属权利要求，并且没有按照细则6.4(a)第2句和第3句的要求撰写。

CSPC Exhibit 1003

国际检索报告
关于同族专利的信息

国际申请号
PCT/CN2016/073844

检索报告引用的专利文件			公布日 (年/月/日)	同族专利			公布日 (年/月/日)
WO	2014152199	A1	2014年 9月 25日	KR	20150132864	A	2015年 11月 26日
				AU	2014240012	A1	2015年 9月 24日
				IL	241004	D0	2015年 11月 30日
				SG	11201507432X	A	2015年 10月 29日
				CN	105209076	A	2015年 12月 30日
				EP	2968589	A1	2016年 1月 20日
				CA	2906022	A1	2014年 9月 25日
				US	2014286968	A1	2014年 9月 25日
				TW	201519904	A	2015年 6月 1日
CN	103772504	A	2014年 5月 7日		无		

CSPC Exhibit 1003

Electronically Filed

PRELIMINARY AMENDMENT Under 37 CFR § 1.115 Address to: Mail Stop Amendment Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Attorney Docket No.	CCPIT-002
	Confirmation No.	TBD
	First Named Inventor	Hu, Chaohong
	Application Number	TBD
	Filing Date	HEREWITH
	Group Art Unit	TBD
	Examiner Name	TBD
	Title: *"Antibody-Drug Conjugate"*	

Sir:

Prior to the examination of the above-referenced application on the merits, please enter the amendments below.

AMENDMENTS TO THE SPECIFICATION

Please insert the following paragraph before the heading "Technical Field" on page 1:

Cross-Reference to Related Applications

This application is a § 371 national phase of International Application No. PCT/CN2016/073844, filed on February 16, 2016, which claims the benefit of Chinese Application No. 201510085038.8, filed on February 17, 2015, which applications are incorporated by reference herein.

CSPC Exhibit 1003
Page 95 of 459

AMENDMENTS TO THE CLAIMS

A complete listing of the claims in this case, with their status, is shown below.

1. (Canceled)

2. (Currently Amended) **An antibody-drug conjugate, a pharmaceutically acceptable salt thereof, a solvate thereof or a solvate of the salt, comprising an anti-epidermal growth factor receptor antibody covalently linked to a cytotoxic agent**~~The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to claim 1~~, wherein the anti-epidermal growth factor receptor antibody comprises a heavy chain and a light chain, wherein the heavy chain has a variable region comprising CDR1, CDR2 and CDR3 having sequences as shown in SEQ ID NOs: 5 to 7 or mutants thereof, and the light chain has a variable region comprising CDR1, CDR2 and CDR3 having sequences as shown in SEQ ID NOs: 12 to 14 or mutants thereof.

3. (Original) The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to claim 2, wherein FR1, FR2, FR3, FR4 of the variable region of the heavy chain of the anti-epidermal growth factor receptor antibody respectively comprise sequences as shown in SEQ ID NOs: 8 to 11 or mutants thereof.

4. (Original) The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to claim 2, wherein FR1, FR2, FR3, FR4 of the variable region of the light chain of the anti-epidermal growth factor receptor antibody respectively comprise sequences as shown in SEQ ID NOs: 15 to 18, or mutants thereof.

5. (Currently Amended) The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to ~~any one of claims 1 to 4~~**claim 2**, wherein the heavy chain of the anti-epidermal growth factor receptor antibody has a constant region selected from the group consisting of a human IgG constant region, a human IgM constant region, a human IgA constant region, a human IgD constant region and a mutant thereof.

6. (Original) The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to claim 5, wherein the IgG is selected from the group consisting of IgG1, IgG2, IgG3 and IgG4.

7. (Currently Amended) The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to claim 5, wherein the constant region of the heavy chain of the anti-epidermal growth factor receptor antibody comprises an amino acid sequence as shown in SEQ ID NO: 3, or comprises a sequence with an identity of greater than 70%, ~~for example,~~**or** greater than 75%, 80%, 85%, 90%, 95%, or 99% to the sequence as shown in SEQ ID NO: 3.

8. (Currently Amended) The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to ~~any one of claims 1 to 7~~**claim 2**, wherein the light chain of the anti-epidermal growth factor receptor antibody has a constant region selected from the group consisting of a human lambda constant region, a human kappa constant region and a mutant thereof.

9. (Currently Amended) The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to claim 8, wherein the constant region of the light chain of the anti-epidermal growth factor receptor antibody comprises an amino acid sequence as shown in SEQ ID NO: 4, or comprises a sequence with an identity of greater than 70%, ~~for example,~~**or** greater than 75%, 80%, 85%, 90%, 95%, or 99% to the sequence as shown in SEQ ID NO: 4.

10. (Currently Amended) The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to ~~any one of claims 1 to 9~~**claim 2**, which has a structure as shown in Formula I,

$$Ab\text{-}(L\text{-}D)_p$$

Formula I

wherein:

Ab represents the anti-epidermal growth factor receptor antibody;

L represents a linker;

D represents the cytotoxic agent;

p represents 1-9~~, for example 2-6, e.g. 3-5~~.

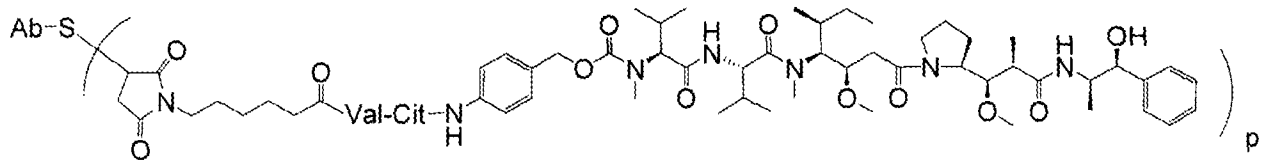
11. (Currently Amended) The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to ~~any one of claims 1 to 10~~**claim 10**, wherein the cytotoxic agent is selected from the group consisting of chemotherapeutic agents, toxins, radioisotopes, cytokines, antibiotics, enzymes, nanoparticles and biologically active peptides.

12. (Currently Amended) The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to claim 11, wherein the cytotoxic agent is selected from the group consisting of Monomethyl auristatin E (MMAE), Monomethyl auristatin F (MMAF), maytansinoid alkaloids~~ (e.g., Maytansine DM1, Maytansine DM4)~~, Calicheamicin, duocarmycin MGBA, doxorubicin, ricin, diphtheria toxin and other toxins, I131, interleukins, tumor necrosis factors, chemokines and nanoparticles~~, etc., preferably MMAE~~.

13. (Original) The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to claim 10, wherein the linker is cleavable or non-cleavable.

14. (Original) The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to claim 13, wherein the linker is selected from the group consisting of 6-maleimidocaproyl (MC), maleimidopropionyl (MP), valine-citrulline (val-cit), alanine-phenylalanine (ala-phe), p-aminobenzyloxycarbonyl (PAB), N-succinimidyl 4-(2-pyridylthio)valerate (SPP), N-succinimidyl 4-(N-maleimidomethyl) -cyclohexane-1-carboxylate (SMCC), N-succinimidyl (4-iodo-acetyl) aminobenzoate (SIAB), and 6-maleimidocaproyl-valine-citrulline-p-aminobenzyloxycarbonyl (MC-vc-PAB), preferably 6-maleimidocaproyl-valine-citrulline-p-aminobenzyloxycarbonyl (MC-vc-PAB).

15. (Currently Amended) The antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to ~~any one of claims 1 to 14~~**claim 10**, which is:



wherein, Ab represents the anti-epidermal growth factor receptor antibody, p is 1-8~~, e.g. 2-6, e.g. 3-5~~.

16. (Currently Amended) A composition, which comprises the antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to ~~any one of claims 1 to 15~~**claim 2**, optionally, further comprises at least one pharmaceutically acceptable carrier, diluent or excipient.

17.-22. (Canceled)

23. (Currently Amended) A method for prophylaxis and/or treatment of a disease associated with epidermal growth factor receptor (EGFR), wherein the method comprises a step of administering to a subject in need a prophylactically and/or therapeutically effective amount of the antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to ~~any one of claims 1 to 15~~**claim 2**.

24. (Currently Amended) The method according to claim 23, wherein the disease associated with epidermal growth factor receptor (EGFR) is ~~a tumor associated with EGFR, such as~~ a tumor associated with overexpression of EGFR~~, e.g., selected from colon cancer, rectal cancer, head and neck cancer, lung cancer, ovarian cancer, cervical cancer, bladder cancer, esophageal cancer, breast cancer, renal cancer, prostate cancer, gastric cancer, pancreatic cancer and brain glioma~~.

25. (Currently Amended) A method for inhibiting tumor angiogenesis, delaying tumor progression, inhibiting tumor growth, or inhibiting tumor cell proliferation, wherein the method comprises a step of administering to a subject in need a prophylactically and/or therapeutically effective amount of the antibody-drug conjugate, the pharmaceutically acceptable salt thereof, the solvate thereof or the solvate of the salt according to ~~any one of claims 1 to 15~~**claim 2**.

26. (Original) The method according to claim 25, wherein the tumor is selected from colon cancer, rectal cancer, head and neck cancer, lung cancer, ovarian cancer, cervical cancer, bladder cancer, esophageal cancer, breast cancer, renal cancer, prostate cancer, gastric cancer, pancreatic cancer and brain glioma.

27. (Currently Amended) The method according to claim 24~~ or 26~~, wherein the tumor is a tumor with KRAS gene mutation~~, for example, colon cancer, colorectal cancer, lung cancer or pancreatic cancer with KRAS gene mutation~~.

28. (Currently Amended) The method according to claim 24~~ or 26~~, wherein the tumor is a tumor with BRAF gene mutation~~, for example colon cancer, rectal cancer and lung cancer with BRAF gene mutation~~.

29.-34. (Canceled)

35. (New) The method according to claim 26, wherein the tumor is a tumor with KRAS gene mutation.

36. (New) The method according to claim 26, wherein the tumor is a tumor with BRAF gene mutation.

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Page 100 of 459

REMARKS UNDER 37 CFR § 1.115

Formal Matters

Claims 2-16, 23-28, 35 and 36 are pending after entry of the amendments set forth herein.

Claims 1, 17-22 and 29-34 are canceled without prejudice.

Claims 2, 5, 7-12, 15, 16, 23-25, 27 and 28 are amended.

Please replace the claims currently on file with the claim set provided above.

No new matter has been added.

Certification Regarding Sequence Listing

Applicant submits herewith the attached Sequence Listing in .txt format. As set out in MPEP §2422.03(a), the Office has advised that if the sequence listing text file submitted via EFS-Web complies with the requirements of 37 CFR 1.824(a)(2)-(6) and (b) (i.e., is a compliant sequence listing ASCII text file), the text file will serve as both the paper copy required by 37 CFR 1.821(c) and the computer readable form (CRF) required by 37 CFR 1.821(e). Further, per MPEP §2422.03(a), neither (1) a second copy of the sequence listing in a PDF file; nor (2) a statement under 37 CFR 1.821(f) (indicating that the paper copy and CRF copy of the sequence listing are identical) should be submitted.

The Sequence Listing was prepared with the software PatentIn version 3.2, and conforms to the Patent Office guidelines. Applicant respectfully submits that the subject application is now in compliance with 37 CFR §§ 1.821-1.825. I hereby certify that the enclosed submission includes no new matter.

Applicant submits that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: August 14, 2017

By: /James S. Keddie/
James S. Keddie
Registration No. 48,920

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Redwood City, California 94065
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PTO/SB/08a (03-15)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	
Filing Date	
First Named Inventor	Hu, Chaohong
Art Unit	
Examiner Name	
Attorney Docket Number	CCPIT-002

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
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FOREIGN PATENT DOCUMENTS Remove

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	WO 2014152199	WO		2014-09-25	ABBVIE INC		
	2	CN 103772504	CN		2014-05-07	SHANGHAI JMT BIOTECHNOLOGY CO LTD		

If you wish to add additional Foreign Patent Document citation information please click the Add button Add

NON-PATENT LITERATURE DOCUMENTS Remove

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	
	Filing Date	
	First Named Inventor	Hu, Chaohong
	Art Unit	
	Examiner Name	
	Attorney Docket Number	CCPIT-002

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	1		

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EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	
	Filing Date	
	First Named Inventor	Hu, Chaohong
	Art Unit	
	Examiner Name	
	Attorney Docket Number	CCPIT-002

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

See attached certification statement.

The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/James S. Keddie/	Date (YYYY-MM-DD)	2017-08-14
Name/Print	James S. Keddie	Registration Number	48920

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 September 2014 (25.09.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/152199 A1

(51) International Patent Classification:
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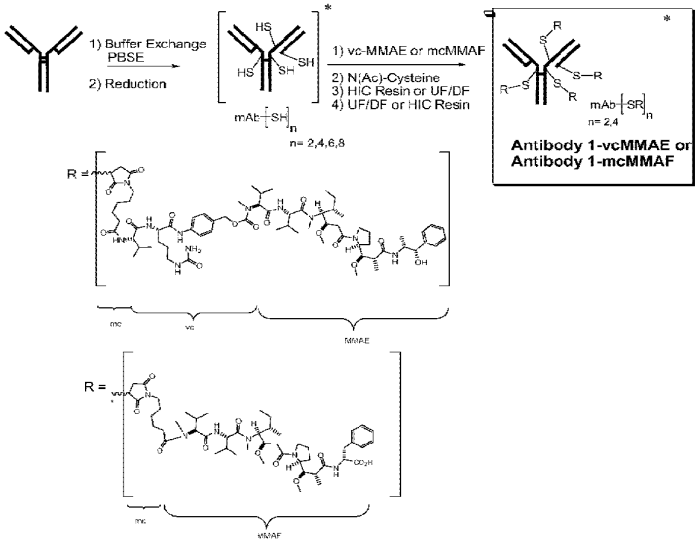
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[Continued on next page]

(54) Title: ANTIBODY DRUG CONJUGATE (ADC) PURIFICATION

Figure 1



(57) Abstract: The invention provides methods for obtaining compositions having antibody drug conjugates (ADCs) with specified drug to antibody ratios (DARs). Included in the invention is a method for purifying an ADC mixture having ADCs with a drug loaded species of 6 or more by contacting the mixture with a hydrophobic resin such that a composition comprising less than 15% of the 6 or more drug loaded species is obtained. The invention also provides a composition wherein 70% or more of the ADCs present have a drug loaded species of 4 or less, wherein the ADC comprises an anti-EGFR antibody and an auristatin.

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ANTIBODY DRUG CONJUGATE (ADC) PURIFICATION

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 61/792,834, filed on March 15, 2013. The contents of the aforementioned priority document are hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

Antibody drug conjugates (ADC) are an emerging class of potent anti-cancer agents, which have recently demonstrated remarkable clinical benefit. ADCs are comprised of a cytotoxic agent attached to an antibody via a stable linker. Putatively, by a series of events, including antigen binding at the cell surface, endocytosis, trafficking to the lysosome, ADC degradation, release of payload, interruption of cellular processing (e.g. mitosis) and apoptosis, ADCs may destroy cancer cells possessing an over-expression of cell-surface proteins. ADCs combine the antigen-driven targeting properties of monoclonal antibodies with the potent anti-tumor effects of cytoxic agents. For example, in 2011 ADCETRIS® (an anti-CD30 antibody-MMAE ADC) gained regulatory approval for the treatment of refractory Hodgkin lymphoma and systemic anaplastic lymphoma.

Studies have demonstrated deleterious effects of a high drug loaded ADCs (Liu *et al.* (2010) *Analy. Chem.* 82:5219). These deleterious effects of higher levels of conjugation include increased propensity towards aggregate formation (King *et al.* (2002) *J Med. Chem.* 45:4336; Hollander *et al.* (2008) *Bioconjugate Chem* 19:358; Burke *et al.* (2009) *Bioconjugate Chem* 20:1242; and Zhao *et al.* (2011) *J Med. Chem.* 54:3606).

Controlling the drug load of an ADC has been attempted using various methods, including: (i) limiting the molar excess of drug-linker intermediate or linker reagent relative to antibody, (ii) limiting the conjugation reaction time or temperature, and (iii) partial or limiting reductive conditions for cysteine thiol modification. While reduction methods that limit the number of attachment sites on the antibody have been used to achieve ADCs with fewer drugs per antibody (Alley *et al.* (2004) *Proc Amer Assoc Cancer Res* 45:Abst 627), there remains a need for methods and compositions that can provide optimal drug loaded species.

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SUMMARY OF INVENTION

The invention provides effective methods for separating antibody drug conjugates (ADCs) having different drug loads, as well as compositions obtained using such methods. The invention also provides compositions where higher drug load species of ADCs are removed.

The invention features, in one embodiment, a method of obtaining a composition comprising Antibody Drug Conjugates (ADCs), said method comprising contacting an ADC mixture comprising a drug loaded species of 4 or less and a drug loaded species of 6 or more with a hydrophobic resin, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug loaded species of 4 or less; and removing the hydrophobic resin from the ADC mixture, such that the composition comprising ADCs is obtained, wherein the composition comprises less than 15% of the drug loaded species of 6 or more, and wherein the ADC comprises an antibody conjugated to an auristatin. In one embodiment, the composition comprises less than 10% of the drug loaded species of 6 or more. In another embodiment, the composition comprises 5% or less of the drug loaded species of 6 or more.

In one embodiment, the method of the invention includes the use of a hydrophobic resin weight which is 3 to 12 times the weight of the drug loaded species of 6 or more in the ADC mixture. In one embodiment, the hydrophobic resin weight is 4 to 8 times the weight of the drug loaded species of 6 or more in the ADC mixture. In a further embodiment, the hydrophobic resin weight is 5 to 10 times the weight of the drug loaded species of 6 or more in the ADC mixture.

In yet another embodiment, the hydrophobic resin weight is 6 to 12 times the weight of the drug loaded species of 6 or more in the ADC mixture, and wherein the ADC mixture comprises between 0 to 1 N NaCl, or an equivalent ionic strength thereof. In a further embodiment, the hydrophobic resin weight is 3 to 6 times the weight of the drug loaded species of 6 or more in the ADC mixture, and wherein the ADC mixture comprises between 1 to 2N NaCl, or an equivalent ionic strength thereof. In yet another embodiment, the hydrophobic resin weight is 3 to 7 times the weight of the drug loaded species of 6 or more in the ADC mixture, and wherein the auristatin is monomethylauristatin E (MMAE). In a

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further embodiment, the hydrophobic resin weight is 5 to 10 times the weight of the drug loaded species of 6 or more in the ADC mixture, and wherein the auristatin is monomethylauristatin F (MMAF). In a further embodiment, the hydrophobic resin weight is 3 to 7 times the weight of the drug loaded species of 6 or more in the ADC mixture, and wherein the auristatin is monomethylauristatin E (MMAE).

The invention further provides a method of producing a composition comprising ADCs with an average Drug-to-Antibody Ratio (DAR) of 4.5 or less and comprising less than 15% undesired ADCs, said method comprising contacting an ADC mixture with a hydrophobic resin, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the undesired ADCs; and removing the hydrophobic resin from the ADC mixture, such that the composition with an average DAR of 4.5 or less and comprising less than 15% undesired ADCs is produced, wherein the ADC comprises an antibody conjugated to an auristatin. In one embodiment, the composition with an average DAR of 4 or less comprises less than 10% undesired ADCs. In one embodiment, the undesired ADCs are 6 and 8 drug loaded species. In a further embodiment, the undesired ADCs are an 8 drug loaded species.

In one embodiment, the amount of hydrophobic resin added to the ADC mixture is a resin weight which is 3 to 12 times the weight of the undesired ADCs in the ADC mixture.

In a further embodiment, the amount of hydrophobic resin added to the ADC mixture is a resin weight which is 4 to 8 times the weight of the drug loaded species of 6 or more in the ADC mixture. In yet another embodiment, the amount of hydrophobic resin added to the ADC mixture is a resin weight which is 5 to 7 times the weight of the drug loaded species of 6 or more in the ADC mixture. In a further embodiment, the hydrophobic resin weight is 6 to 12 times the weight of the drug loaded species of 6 or more in the ADC mixture and wherein the ADC mixture comprises between 0 to 1 N NaCl, or an equivalent ionic strength thereof. In one embodiment, the hydrophobic resin weight is 3 to 6 times the weight of the drug loaded species of 6 or more in the ADC mixture and the ADC mixture comprises between 1 to 2 N NaCl, or an equivalent ionic strength thereof. In another embodiment, the hydrophobic resin weight is 3 to 7 times the weight of the drug loaded species of 6 or more in the ADC mixture, and wherein the auristatin is monomethylauristatin E (MMAE). In still another embodiment, the hydrophobic resin weight is 5 to 10 times the weight of the drug

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loaded species of 6 or more in the ADC mixture, and wherein the auristatin is monomethylauristatin F (MMAF). In yet a further embodiment, the hydrophobic resin weight is 3 to 7 times the weight of the drug loaded species of 6 or more in the ADC mixture, and wherein the auristatin is monomethylauristatin E (MMAE). In one embodiment, the hydrophobic resin weight is 5 to 10 times the weight of the drug loaded species of 6 or more in the ADC mixture, and wherein the auristatin is monomethylauristatin F (MMAF).

In one embodiment, the method of the invention is used to obtain a composition comprising ADCs with an average DAR of 4.5 or less. In one embodiment, the method of the invention is used to obtain a composition comprising ADCs with an average DAR of 4 or less. In one embodiment, the method of the invention is used to obtain a composition comprising ADCs with an average DAR of 3.5 or less. In one embodiment, the method of the invention is used to obtain a composition comprising ADCs with an average DAR of 3 or less. In one embodiment, the composition has an average DAR of 2.5 or less.

In one embodiment, the method of the invention features adding a hydrophobic resin to an ADC mixture to form a resin mixture, wherein the resin mixture has an ionic strength which is equal to or higher than the ADC mixture.

In a further embodiment of the invention, the ADC mixture was obtained following an ultrafiltration /diafiltration process.

In one embodiment of the invention, the hydrophobic resin used in the methods of the invention is a butyl hydrophobic resin.

In one embodiment of the invention, the method of the invention is a batch process or, alternatively, a circulation process or a flow through process.

In one embodiment, the invention features a composition obtained using the methods described herein.

The invention further features a composition comprising ADCs, wherein 70% of the ADCs have a drug loaded species of 4 or less, wherein the ADC comprises an anti-EGFR antibody (e.g., antibody 1) and an auristatin (e.g., MMAE or MMAF). In one embodiment of the invention, the composition comprises 75% ADCs present having a drug loaded species of 4 or less. In another embodiment of the invention, the composition comprises 80% ADCs present having a drug loaded species of 4 or less. In one embodiment of the invention, the composition comprises 85% ADCs present having a drug loaded species of 4 or less. In a

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further embodiment of the invention, the composition comprises 90% ADCs having a drug loaded species of 4 or less. In one embodiment, the composition of the invention comprises 95% ADCs present having a drug loaded species of 4 or less. In another embodiment, the composition of the invention comprises ADCs wherein 70% or more of the ADCs have a drug loaded species of 4 to 1, 3 to 1, or, alternatively, 2 to 1.

The invention further features a composition comprising ADCs with an average DAR of 4.5 or less, wherein the ADC comprises an anti-EGFR antibody (e.g., antibody 1) and an auristatin (e.g., MMAE or MMAF). In one embodiment of the invention, the composition comprises ADCs having an average DAR of 4 or less. In another embodiment of the invention, the composition comprises ADCs having an average DAR of 4 or less. In yet another embodiment of the invention, the composition comprises ADCs having an average DAR of 3.5 or less. In one embodiment of the invention, the composition comprises ADCs having an average DAR of 3 or less. In yet another embodiment of the invention, the composition comprises ADCs having an average DAR of 2.5 or less. In another embodiment, the composition of the invention comprises anti-EGFR ADCs (e.g., antibody 1 conjugated to MMAE or MMAF) with an average DAR of 4.5 to 0.001, 4 to 0.001, 3.5 to 0.001, 3 to 0.001, or, alternatively, 2.5 to 0.001.

In one embodiment, the methods and compositions of the invention include an ADC comprising an anti-Epidermal Growth Factor Receptor (EGFR) antibody. In one embodiment of the invention, the anti-EGFR antibody comprises a light chain variable region comprising a Complementarity Determining Region 1 (CDR1), CDR2, and CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9, respectively, and comprises a heavy chain variable region comprising a CDR1, CDR2, and CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4. In another embodiment of the invention, the anti-EGFR antibody comprises a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 6 and a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 1.

In another embodiment of the invention, the anti-EGFR ADC comprises CDRs (*i.e.,* light chain CDR1, CDR2, and CDR3) described in the light chain variable region set forth in

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the amino acid sequence of SEQ ID NO: 6, and CDRs (*i.e.*, heavy chain CDR1, CDR2, and CDR3) described in the amino acid sequence of SEQ ID NO: 1.

In a further embodiment, the methods and compositions of the invention feature an auristatin which is either monomethylauristatin E (MMAE) or monomethylauristatin F (MMAF).

In one embodiment, the MMAE is conjugated to the antibody via a valine-citrulline (vc) linker (vc-MMAE). In another embodiment, the MMAF is conjugated to the antibody via a maleimidocaproyl linker (mc-MMAF).

In one embodiment, the composition of the invention is a pharmaceutical composition.

Also included in the invention are methods of treating cancer in a subject comprising administering a composition described herein to the subject such that cancer is treated. In one embodiment, the cancer is selected from the group consisting of squamous tumors (including, squamous tumors of the lung, head and neck, cervical, etc.), glioblastoma, glioma, non-small cell lung cancer, lung cancer, colon cancer, head and neck cancer, breast cancer, squamous cell tumors, anal cancer, skin cancer, and vulvar cancer.

In one embodiment, the compositions of the invention are used to treat glioblastoma multiforme.

In one embodiment, the compositions of the invention are used to treat a solid tumor having overexpression of EGFR. In one embodiment, the compositions of the invention are used to treat a subject having an advanced solid tumor likely to overexpress EGFR.

In one embodiment, the compositions of the invention are administered intravenously.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides an overview of the processes described in the examples, including reduction of Antibody 1, conjugation of Antibody 1 to vcMMAE, and purification of the ADC using batch purification with HIC resin.

Figure 2 graphically depicts HIC HPLC analysis of an Antibody 1 ADC solution before and after HIC resin batch purification.

Figure 3 provides an overview of the process described in Example 6 for the purification of ADC mixtures with average DARs of 2.7, 4, and 5.5.

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DETAILED DESCRIPTION

I. Definitions

In order that the present invention may be more readily understood, certain terms are first defined. In addition, it should be noted that whenever a value or range of values of a parameter are recited, it is intended that values and ranges intermediate to the recited values are also intended to be part of this invention.

The term "antibody-drug-conjugate" or "ADC" refers to a binding protein, such as an antibody or antigen binding fragment thereof, chemically linked to one or more chemical drug(s) (also referred to herein as agent(s)) that may optionally be therapeutic or cytotoxic agents. In a preferred embodiment, an ADC includes an antibody, a cytotoxic or therapeutic drug, and a linker that enables attachment or conjugation of the drug to the antibody. An ADC typically has anywhere from 1 to 8 drugs conjugated to the antibody, including drug loaded species of 2, 4, 6, or 8. Non-limiting examples of drugs that may be included in the ADCs are mitotic inhibitors, antitumor antibiotics, immunomodulating agents, vectors for gene therapy, alkylating agents, antiangiogenic agents, antimetabolites, boron-containing agents, chemoprotective agents, hormones, antihormone agents, corticosteroids, photoactive therapeutic agents, oligonucleotides, radionuclide agents, topoisomerase inhibitors, tyrosine kinase inhibitors, and radiosensitizers.

The terms "anti-Epidermal Growth Factor antibody drug conjugate," "anti-EGFR antibody drug conjugate," or "anti-EGFR ADC", used interchangeably herein, refer to an ADC comprising an antibody that specifically binds to EGFR, whereby the antibody is conjugated to one or more chemical agent(s). In one embodiment, the anti-EGFR antibody drug conjugate is Antibody 1 conjugated to an auristatin, e.g., MMAE or MMAF. Amino acid sequences corresponding to the light and heavy chains of Antibody 1 are provided in SEQ ID NOs: 1-10.

The term "auristatin", as used herein, refers to a family of antimitotic agents. Auristatin derivatives are also included within the definition of the term "auristatin". Examples of auristatins include, but are not limited to, auristatin E (AE), monomethylauristatin E (MMAE), monomethylauristatin F (MMAF), and synthetic analogs of dolastatin.

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The term "drug-to-antibody ratio" or "DAR" refers to the number of drugs, e.g., auristatin, attached to the antibody of the ADC. The DAR of an ADC can range from 1 to 8, although higher loads, e.g., 10, are also possible depending on the number of linkage site on an antibody. The term DAR may be used in reference to the number of drugs loaded onto an individual antibody, or, alternatively, may be used in reference to the average or mean DAR of a group of ADCs.

The term "undesired ADC species", as used herein, refers to any drug loaded species which is to be separated from an ADC species having a different drug load. In one embodiment, the term undesired ADC species may refer to drug loaded species of 6 or more, *i.e.*, ADCs with a DAR of 6 or more, including DAR6, DAR7, DAR8, and DAR greater than 8 (*i.e.*, drug loaded species of 6, 7, 8, or greater than 8). In a separate embodiment, the term undesired ADC species may refer to drug loaded species of 8 or more, *i.e.*, ADCs with a DAR of 8 or more, including DAR8, and DAR greater than 8 (*i.e.*, drug loaded species of 8, or greater than 8).

The term "ADC mixture", as used herein, refers to a composition containing a heterogeneous DAR distribution of ADCs. In one embodiment, an ADC mixture contains ADCs having a distribution of DARs of 1 to 8, *e.g.*, 2, 4, 6, and 8 (*i.e.*, drug loaded species of 2, 4, 6, and 8). Notably, degradation products may result such that DARs of 1, 3, 5, and 7 may also be included in the mixture. Further, ADCs within the mixture may also have DARs greater than 8. The ADC mixture results from interchain disulfide reduction followed by conjugation. In one embodiment, the ADC mixture comprises both ADCs with a DAR of 4 or less (*i.e.*, a drug loaded species of 4 or less) and ADCs with a DAR of 6 or more (*i.e.*, a drug loaded species of 6 or more).

As used herein, the term "hydrophobic resin" or "hydrophobic interaction resin" refers to a medium consisting of hydrophobic ligands used for purposes of purifying a mixture of molecules, wherein the presence of hydrophobic surface moieties on the molecules within the mixture facilitates an interaction with the medium such that interacting molecules are at least transiently bound to the medium. In one embodiment, the hydrophobic resin is a resin comprising alkyl moieties, *e.g.*, a C_4-C_8 alkyl hydrophobic resin, which is a resin comprising a four to eight straight or branched chain carbon membered alkane radical group such as butyl, pentyl, hexyl, heptyl, or octyl group coupled to a solid

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support (e.g., agarose, silica, etc.). Examples of hydrophobic alkyl resins include a hydrophobic butyl resin or a hydrophobic hexyl resin. In one embodiment, the hydrophobic resin is a resin comprising aryl moieties, *e.g.,* a hydrophobic phenyl resin. In one embodiment, the hydrophobic resin comprises an alkenyl moiety. In one embodiment, the hydrophobic resin comprises an ether moiety. In yet another embodiment, the hydrophobic resin comprises a phenyl moiety. The hydrophobic moieties (e.g., alkyl, aryl, etc.) may be linked to an inert substance (e.g., silica, agarose, and/or other polysaccharide polymers). In one embodiment, the resin is a methacrylate resin.

The term "ionic strength" broadly refers to a measure of the concentration of ions in a solution, *i.e.*, the conductivity of a solution. Exemplary salts that may be used to modulate the ionic strength of a solution include, but are not limited to sodium bromide, sodium chloride, sodium citrate, sodium iodide, sodium phosphate, sodium sulfate, potassium bromide, potassium chloride, potassium citrate, potassium iodide, potassium phosphate, potassium sulfate, cesium chloride, lithium chloride, or other salts of ammonia (e.g., NH_4Cl, $(NH_4)_2SO_4$), carbonates ($NaHCO_3$), citric acid ($NaH_2(C_3H_5O(COO)_3)$, $Na_2H(C_3H_5O(COO)_3)$, $Na_3H(C_3H_5O(COO)_3)$), phosphoric acid (e.g., KH_2PO_4, K_2HPO_4 , K_3PO_4), nitrates (KNO_3), or any mixture of these components. Those skilled in the art appreciate that both the anion and the cation can be varied as is known to the person skilled in the art, as long as sufficient ionic strength is provided without precipitation or other undesired side-effects.

The term "anti-EGFR antibody" is meant to refer to an antibody that specifically binds to EGFR. An antibody "which binds" an antigen of interest, *i.e.*, EGFR, is one capable of binding that antigen with sufficient affinity such that the antibody is useful in targeting a cell expressing the antigen. Antibody 1 is an example of an anti-EGFR antibody.

The term "antibody" broadly refers to an immunoglobulin (Ig) molecule, generally comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains, or any functional fragment, mutant, variant, or derivative thereof, that retains the essential target binding features of an Ig molecule.

In a full-length antibody, each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light

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chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY) and class (e.g., IgG1, IgG2, IgG 3, IgG4, IgA1 and IgA2) or subclass.

The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.*, hIL-13). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Such antibody embodiments may also be bispecific, dual specific, or multi-specific formats; specifically binding to two or more different antigens. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546, Winter et al., PCT publication WO 90/05144 A1 herein incorporated by reference), which comprises a single variable domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the

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two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see *e.g.*, Holliger, P., *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Poljak, R.J., *et al.* (1994) *Structure* 2:1121-1123). Such antibody binding portions are known in the art (Kontermann and Dubel eds., Antibody Engineering (2001) Springer-Verlag. New York. 790 pp. (ISBN 3-540-41354-5).

An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds EGFR is substantially free of antibodies that specifically bind antigens other than EGFR). An isolated antibody that specifically binds EGFR may, however, have cross-reactivity to other antigens, such as EGFR molecules from other species. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

The term "humanized antibody" refers to antibodies which comprise heavy and light chain variable region sequences from a non-human species (*e.g.*, a mouse) but in which at least a portion of the VH and/or VL sequence has been altered to be more "human-like", *i.e.*, more similar to human germline variable sequences. In a particular embodiment, the term "humanized antibody" refers to an antibody or antibody variant, derivative or fragment, which specifically binds to an antigen of interest, and comprises a framework (FR) region having substantially the amino acid sequence of a human antibody, and comprises CDRs having substantially the amino acid sequence of a non-human antibody. As used herein, the term "substantially" in the context of a CDR refers to a CDR having an amino acid sequence at least 80%, preferably at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of a non-human antibody CDR. In one embodiment, one type of humanized antibody is a CDR-grafted antibody, in which human CDR sequences are introduced into non-human VH and VL sequences to replace the corresponding nonhuman CDR sequences.

As used herein, the term "CDR" refers to the complementarity determining region within antibody variable sequences. There are three CDRs in each of the variable regions of the heavy chain and the light chain, which are designated CDR1, CDR2 and CDR3, for each of the variable regions. The term "CDR set" as used herein refers to a group of three CDRs that occur in a single variable region capable of binding the antigen. The exact boundaries of

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these CDRs have been defined differently according to different systems. The system described by Kabat (Kabat et al., Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987) and (1991)) not only provides an unambiguous residue numbering system applicable to any variable region of an antibody, but also provides precise residue boundaries defining the three CDRs. These CDRs may be referred to as Kabat CDRs. Chothia and coworkers (Chothia &Lesk, J. Mol. Biol. 196:901-917 (1987) and Chothia et al., Nature 342:877-883 (1989)) found that certain sub- portions within Kabat CDRs adopt nearly identical peptide backbone conformations, despite having great diversity at the level of amino acid sequence. These sub-portions were designated as L1, L2 and L3 or H1, H2 and H3 where the "L" and the "H" designates the light chain and the heavy chains regions, respectively. These regions may be referred to as Chothia CDRs, which have boundaries that overlap with Kabat CDRs. Other boundaries defining CDRs overlapping with the Kabat CDRs have been described by Padlan (FASEB J. 9:133-139 (1995)) and MacCallum (J Mol Biol 262(5):732-45 (1996)). Still other CDR boundary definitions may not strictly follow one of the above systems, but will nonetheless overlap with the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. The methods used herein may utilize CDRs defined according to any of these systems, although preferred embodiments use Kabat or Chothia defined CDRs.

The term "disorder" refers to any condition that would benefit from treatment with the formulations of the invention, e.g. a disorder requiring treatment with the anti-EGFR antibody in the formulation. This includes chronic and acute disorders or diseases including those pathological conditions that predispose the subject to the disorder in question.

The term "cancer" is meant to refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include glioblastoma, non-small cell lung cancer, lung cancer, colon cancer, head and neck cancer, breast cancer, squamous cell tumors, anal cancer, skin cancer, and vulvar cancer. In one embodiment, the compositions of the invention are administered to a patient having a tumor(s) containing

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amplifications of the EGFR gene, whereby the tumor expresses the truncated version of the EGFR de2-7. In one embodiment, the formulation of the invention comprising ADC-1 may be administered to a subject for the treatment of colorectal cancer, head and neck cancer (including, but not limited to, hypopharyngeal cancer, oropharyngeal cancer, esophageal cancer, laryngeal cancer, and oral cavity cancer), non-small cell lung cancer, pancreatic cancer, gastric cancer, and breast cancer. More particular examples of such cancers include squamous tumors (including, squamous tumors of the lung, head and neck, cervical, etc.), glioblastoma, glioma, non-small cell lung cancer, lung cancer, colon cancer, head and neck cancer, breast cancer, squamous cell tumors, anal cancer, skin cancer, and vulvar cancer. In one embodiment of the invention, the composition is used to treat a subject having a solid tumor, *e.g.,* a solid tumor likely to over-express the Epidermal Growth Factor Receptor (EGFR), or glioblastoma multiforme.

The term "administering" as used herein is meant to refer to the delivery of a substance (e.g., an anti-EGFR antibody drug conjugate) to achieve a therapeutic objective (e.g., the treatment of an EGFR- associated disorder). Modes of administration may be parenteral, enteral and topical. Parenteral administration is usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

The term "therapeutically effective amount" or "effective amount" of an antibody as used herein refers to an amount effective in the prevention or treatment or alleviation of a symptom of a disorder for the treatment of which the antibody is effective.

The term "treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those patients in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented.

Various aspects of the invention are described in further detail in the following subsections.

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II. Methods for Purifying Antibody Drug Conjugates and Compositions Thereof

The invention provides a method for purifying antibody drug conjugates (ADCs), and provides an effective means for removing undesired species of ADC, *e.g.*, drug loaded species of 6 or more, from a mixture of ADCs. While the methods of the invention may be used to separate any drug loaded species, in a preferred embodiment, the methods described herein are used to separate high drug loaded ADCs from ADCs having optimal drug to antibody ratios (DARs), *e.g.* a DAR of 4 or less. In certain embodiments, the methods of the invention may provide numerous advantages over traditional column chromatography, including improved recovery, as fractionation and subsequent pooling of fractions may not be necessary. It should be understood that the methods and compositions described throughout may be used to purify anti-EGFR antibody-auristatin ADCs, particularly, in a certain embodiment, anti-EGFR ADCs comprising Antibody 1 either coupled via a maleimidocaproyl linker to MMAF (mc-MMAF) or coupled via a maleimidocaproyl valine-citrulline linker to MMAE (vc-MMAE).

The method of the invention generally includes adding a hydrophobic resin to an ADC mixture such that undesired ADCs, *i.e.*, higher drug loaded ADCs, bind the resin and can be selectively removed from the mixture. In certain embodiments, separation of the ADCs may be achieved by contacting an ADC mixture (*e.g.*, a mixture comprising a drug loaded species of ADC of 4 or less and a drug loaded species of ADC of 6 or more) with a hydrophobic resin, wherein the amount of resin is sufficient to allow binding of the drug loaded species which is being removed from the ADC mixture. The resin and ADC mixture are mixed together, such that the ADC species being removed (*e.g.*, a drug loaded species of 6 or more) binds to the resin and can be separated from the other ADC species in the ADC mixture. The amount of resin used in the method is based on a weight ratio between the species to be removed and the resin, where the amount of resin used does not allow for significant binding of the drug loaded species that is desired. Thus, the invention provides methods for reducing the average DAR of an ADC mixture from, for example, 5.5 to less than 4. Further, the purification methods described herein may be used to isolate ADCs having any desired range of drug loaded species, *e.g.*, a drug loaded species of 4 or less, a drug loaded species of 3 or less, a drug loaded species of 2 or less, a drug loaded species of 1 or less.

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The invention provides a purification method whereby a certain species of molecule(s) binds to a surface based on hydrophobic interactions between the species and a hydrophobic resin. In one embodiment, method of the invention refers to a purification process that relies upon the intermixing of a hydrophobic resin and a mixture of ADCs, wherein the amount of resin added to the mixture determines which species (*e.g.*, ADCs with a DAR of 6 or more) will bind.

Following production and purification of an antibody from an expression system (e.g., a mammalian expression system), the antibody is reduced and coupled to a drug through a conjugation reaction. The resulting ADC mixture often contains ADCs having a range of DARs, *e.g.,* 1 to 8. In one embodiment, the ADC mixture comprises a drug loaded species of 4 or less and a drug loaded species of 6 or more. According to the methods of the invention, the ADC mixture may be purified using a process, such as, but not limited to, a batch process, such that ADCs having a drug loaded species of 4 or less are selected and separated from ADCs having a higher drug load (e.g., ADCs having a drug loaded species of 6 or more). Notably, the purification methods described herein may be used to isolate ADCs having any desired range of DAR, *e.g.,* a DAR of 4 or less, a DAR of 3 or less, a DAR of 2 or less.

Thus, in one embodiment, the method of the invention comprises contacting an ADC mixture comprising a drug loaded species of 4 or less and a drug loaded species of 6 or more with a hydrophobic resin to form a resin mixture, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug load species of 4 or less; and removing the hydrophobic resin from the ADC mixture, such that the composition comprising ADCs is obtained, wherein the composition comprises less than 15% of the drug loaded species of 6 or more, and wherein the ADC comprises an antibody conjugated to an auristatin. In a separate embodiment, the method of the invention comprises contacting an ADC mixture comprising a drug loaded species of 4 or less and a drug loaded species of 6 or more with a hydrophobic resin to form a resin mixture, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug load species of 4 or less; and removing the hydrophobic resin from the ADC mixture, such that the composition comprising ADCs is obtained, wherein the composition comprises less than 15%

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of the drug loaded species of 6 or more, and wherein the ADC comprises an antibody conjugated to an auristatin, wherein the hydrophobic resin weight is 3 to 12 times the weight of the drug loaded species of 6 or more in the ADC mixture.

The method of the invention provides an effective method of separating low and high DAR ADCs. In one embodiment, the method may be performed using a batch purification method. The batch purification process generally includes adding the ADC mixture to the hydrophobic resin in a vessel, mixing, and subsequently separating the resin from the supernatant. For example, in the context of batch purification, a hydrophobic resin may be prepared in or equilibrated to the desired equilibration buffer. A slurry of the hydrophobic resin may thus be obtained. The ADC mixture may then be contacted with the slurry to adsorb the specific species of ADC(s) to be separated by the hydrophobic resin. The solution comprising the desired ADCs that do not bind to the hydrophobic resin material may then be separated from the slurry, e.g., by filtration or by allowing the slurry to settle and removing the supernatant. The resulting slurry can be subjected to one or more washing steps. In order to elute bound ADCs, the salt concentration can be decreased. In one embodiment, the process used in the invention includes no more than 50 g of hydrophobic resin.

Thus, in one embodiment of the invention, a batch method may be used to contact an ADC mixture comprising a drug loaded species of 4 or less and a drug loaded species of 6 or more with a hydrophobic resin to form a resin mixture, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug load species of 4 or less; and removing the hydrophobic resin from the ADC mixture, such that the composition comprising ADCs is obtained, wherein the composition comprises less than 15% of the drug loaded species of 6 or more, and wherein the ADC comprises an antibody conjugated to an auristatin. In a separate embodiment, a batch method is used to contact an ADC mixture comprising a drug loaded species of 4 or less and a drug loaded species of 6 or more with a hydrophobic resin to form a resin mixture, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug load species of 4 or less; and removing the hydrophobic resin from the ADC mixture, such that the composition comprising ADCs is obtained, wherein the composition comprises less than 15%

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of the drug loaded species of 6 or more, and wherein the ADC comprises an antibody conjugated to an auristatin, wherein the hydrophobic resin weight is 3 to 12 times the weight of the drug loaded species of 6 or more in the ADC mixture.

Alternatively, in a separate embodiment, the invention may be performed using a circulation process, whereby the resin is packed in a container and the ADC mixture is passed over the hydrophobic resin bed until the specific species of ADC(s) to be separated have been removed. The supernatant (containing the desired ADC species) is then pumped from the container and the resin bed may be subjected to washing steps.

A circulation process may be used to contact an ADC mixture comprising a drug loaded species of 4 or less and a drug loaded species of 6 or more with a hydrophobic resin to form a resin mixture, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug load species of 4 or less; and removing the hydrophobic resin from the ADC mixture, such that the composition comprising ADCs is obtained, wherein the composition comprises less than 15% of the drug loaded species of 6 or more, and wherein the ADC comprises an antibody conjugated to an auristatin. In a separate embodiment, a circulation process is used to contact an ADC mixture comprising a drug loaded species of 4 or less and a drug loaded species of 6 or more with a hydrophobic resin to form a resin mixture, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug load species of 4 or less; and removing the hydrophobic resin from the ADC mixture, such that the composition comprising ADCs is obtained, wherein the composition comprises less than 15% of the drug loaded species of 6 or more, and wherein the ADC comprises an antibody conjugated to an auristatin, wherein the hydrophobic resin weight is 3 to 12 times the weight of the drug loaded species of 6 or more in the ADC mixture.

Alternatively, in a separate embodiment of the invention, the purification method may be performed using a flow through process, whereby resin is packed in a container, *e.g.*, a column, and the ADC mixture is passed over the packed resin such that the desired ADC species does not substantially bind to the resin and flows through the resin, and the undesired ADC species is bound to the resin. A flow through process may be performed in a single

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pass mode (where the ADC species of interest are obtained as a result of a single pass through the resin of the container) or in a multi-pass mode (where the ADC species of interest are obtained as a result of multiple passes through the resin of the container). The flow through process is performed such that the weight of resin selected binds to the undesired ADC population, and the desired ADCs (*e.g.*, DAR 2-4) flow over the resin and are collected in the flow through after one or multiple passes.

In one embodiment of the invention, a flow through process may be used to contact an ADC mixture comprising a drug loaded species of 4 or less and a drug loaded species of 6 or more with a hydrophobic resin, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug load species of 4 or less, where the drug load species of 4 or less passes over the resin and is subsequently collected after one or multiple passes, such that the composition comprising the desired ADCs (e.g. DAR 2-4) is obtained, wherein the composition comprises less than 15% of the drug loaded species of 6 or more, and wherein the ADC comprises an antibody conjugated to an auristatin. In a separate embodiment, a flow through process is used to contact an ADC mixture comprising a drug loaded species of 4 or less and a drug loaded species of 6 or more with a hydrophobic resin by passing the ADC mixture over the resin, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug load species of 4 or less, where the drug load species of 4 or less passes over the resin and is subsequently collected, such that the composition comprising ADCs is obtained, wherein the composition comprises less than 15% of the drug loaded species of 6 or more, and wherein the ADC comprises an antibody conjugated to an auristatin, wherein the amount of hydrophobic resin weight is 3 to 12 times the weight of the drug loaded species of 6 or more in the ADC mixture.

In one embodiment of the invention, the resin is washed with a one or more washes following the flow through process in order to further recover ADCs having the desired DAR range (found in the wash filtrate). For example, a plurality of washes having decreasing conductivity may be used to further recover ADCs having the DAR of interest. The elution material obtained from the washing of the resin may be subsequently combined with the

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filtrate resulting from the flow through process for improved recovery of ADCs having the DAR of interest.

The purification methods of the invention are based on the use of a hydrophobic resin to separate high vs. low drug loaded species of ADC. Hydrophobic resin comprises hydrophobic groups which interact with the hydrophobic properties of the ADCs. Hydrophobic groups on the ADC interact with hydrophobic groups within the hydrophobic resin. The more hydrophobic a protein is the stronger it will interact with the hydrophobic resin.

Hydrophobic resin normally comprises a base matrix (e.g., cross-linked agarose or synthetic copolymer material) to which hydrophobic ligands (e.g., alkyl or aryl groups) are coupled. Many hydrophobic resins are available commercially. Examples include, but are not limited to, Phenyl Sepharose™ 6 Fast Flow with low or high substitution (Pharmacia LKB Biotechnology, AB, Sweden); Phenyl Sepharose™ High Performance (Pharmacia LKB Biotechnology, AB, Sweden); Octyl Sepharose™ High Performance (Pharmacia LKB Biotechnology, AB, Sweden); Fractogel™ EMD Propyl or Fractogel™ EMD Phenyl columns (E. Merck, Germany); Macro-Prep™ Methyl or Macro-Prep™. t-Butyl Supports (Bio-Rad, California); WP HI-Propyl (C_3)™ (J. T. Baker, New Jersey); and Toyopearl™ ether, hexyl, phenyl or butyl (TosoHaas, PA). In one embodiment, the hydrophobic resin is a butyl hydrophobic resin. In another embodiment, the hydrophobic resin is a phenyl hydrophobic resin. In another embodiment, the hydrophobic resin is a hexyl hydrophobic resin, an octyl hydrophobic resin, or a decyl hydrophobic resin. In one embodiment, the hydrophobic resin is a methacrylic polymer having n-butyl ligands (*e.g.* TOYOPEARL® Butyl-600M).

The methods of the invention are based, at least in part, on the discovery that a hydrophobic resin may be used in certain amounts to selectively bind to ADCs having certain DARs. The binding between the resin and ADCs having a given DAR is dependent upon the weight of the resin relative to the weight of the ADCs which are to be removed from the ADC mixture. By varying the amount of resin load (calculated based on the dry weight) contacted to the ADC mixture relative to the specific drug load species weight in the ADC mixture, the resin will selectively bind ADCs having, for example, a DAR of 8 or more, ADCs having a DAR of 6-8, ADCs having a DAR of 5-8, etc. Thus, the selectivity of the

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hydrophobic resin is dependent upon the weight ratio of the resin and the weight of the ADC species to be removed by the resin. In one embodiment, the hydrophobic resin weight contacted with the ADC mixture is 3 to 12 times the weight of the drug loaded species of 6 or more in the ADC mixture. In one embodiment, the hydrophobic resin weight contacted with the ADC mixture is 4 to 8 times the weight of the drug loaded species of 6 or more in the ADC mixture. In one embodiment, the hydrophobic resin weight contacted with the ADC mixture is 5 to 10 times the weight of the drug loaded species of 6 or more in the ADC mixture. In another embodiment, the hydrophobic resin weight contacted with the ADC mixture is 5 to 7 times the weight of the drug loaded species of 6 or more in the ADC mixture. In another embodiment, the hydrophobic resin weight contacted with the ADC mixture is 5 to 6 times the weight of the drug loaded species of 6 or more in the ADC mixture. For example, as described in Table 5 in the examples below, about 5-10 weights of resin (dry) were employed to reduce the 6 and 8 loaded drug species (3.2 mg resin / 0.54 mg 6/8 load species = approx. 6), resulting in an enriched composition (enriched for ADCs having DARs of less than 6). In another example, as described in Table 7 below, a resin weight of approximately 8 to 12 times that of the 6 and 8 drug load species was proven to be effective for reducing those species from the ADC mixture. In a further example, as described in Table 7 below, a resin weight of approximately 4 times that of the 6 and 8 drug load species was proven to be effective for significantly reducing those species from the ADC mixture.

The selectivity of the resin for ADCs may be impacted by the ionic strength of the resin mixture in combination with the ratios identified herein as providing appropriate load resin:ADC weight ratios that result in selective binding of ADCs having a certain desired DAR distribution, e.g., a DAR distribution of 6-8. Generally, by decreasing the ionic strength of the resin mixture, the hydrophobic resin will be less adsorbent, whereas an increase in the ionic strength of the resin mixture will provide a more adsorbent resin. Adsorption of ADCs to hydrophobic resin is favored by high salt concentrations, but the actual concentrations may vary over a wide range depending on the nature of the ADC and the particular hydrophobic resin chosen. In general, Na, K or NH_4 sulfates effectively promote ligand-protein interaction in hydrophobic resin. Salts may be formulated that influence the strength of the interaction as given by the following relationship:

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$(NH_4)_2SO_4$>Na_2SO_4>NaCl>NH_4Cl>NaBr>NaSCN. In general, salt concentrations of between about 0.75 and about 2 M ammonium sulfate or between about 1 and 4 M NaCl are useful. In one embodiment, the resin mixture has an ionic strength of 0-2 N NaCl. The ionic strength of the ADC mixture may be adjusted prior to, concurrently with, or following the addition of the hydrophobic resin.

In one embodiment, the method of the invention uses a hydrophobic resin weight which is 6 to 12 times the weight of the drug loaded species of 6 or more in the ADC mixture where the ADC mixture has an ionic strength of 0 to 1 N NaCl, or an equivalent ionic strength thereof. In a separate embodiment, the separation method of the invention is carried out using a hydrophobic resin weight which is 3 to 6 times the weight of the drug loaded species of 6 or more in the ADC mixture, and where the ADC mixture comprises between 1 to 2 N NaCl, or an equivalent ionic strength thereof. The method may also be carried out using a hydrophobic resin weight which is 3 to 7 times the weight of the drug loaded species of 6 or more in the ADC mixture, and wherein the auristatin is monomethylauristatin E (MMAE). An additional method for separating a drug loaded species of 6 or more includes contact of an ADC mixture with a hydrophobic resin weight that is 5 to 10 times the weight of the drug loaded species of 6 or more, wherein the auristatin is monomethylauristatin F (MMAF).

Additional purification or processing steps may be performed prior to or following the methods described herein. For example, in one embodiment, the ADC mixture is obtained following an ultrafiltration /diafiltration process. In another embodiment, the purified composition of ADCs is subjected to ultrafiltration / diafiltration.

In one embodiment, the method of the invention includes contacting an ADC mixture with a hydrophobic resin, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug loaded species of 4 or less, and removing the hydrophobic resin from the ADC mixture. The hydrophobic resin binds the higher drug loaded species, e.g., drug loaded species of 6 or more, while the lower drug loaded species, *e.g.,* the drug loaded species of 4 or less, largely remains in the supernatant. The amount of hydrophobic resin which is contacted with the ADC mixture and does not allow significant binding of the drug loaded species of 4 or less is an amount of resin which, in one

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embodiment, binds 35% or less drug loaded species of 4 or less. In certain embodiments, significant binding of the drug loaded species of 4 or less is defined as 30% or less, 25% or less, 20% or less, 15 % or less, 10% or less, or 5% or less. In other embodiments, significant binding of the drug loaded species is defined as 30% to 1%, 25% to 1%, 20% to 1%, 15% to 1%, 10% to 1%, or 5% to 1%.

In one embodiment, the methods of the invention may be used to obtain compositions having low levels of an undesired ADC species, e.g., a drug loaded species of 6 or more. In one embodiment, the composition of the invention has 15% or less of the drug loaded species of 6 or more. In one embodiment, the composition of the invention has 14% or less of the drug loaded species of 6 or more. In one embodiment, the composition of the invention has 13% or less of the drug loaded species of 6 or more. In one embodiment, the composition of the invention has 12% or less of the drug loaded species of 6 or more. In one embodiment, the composition of the invention has 11% or less of the drug loaded species of 6 or more. In one embodiment, the composition of the invention has 10% or less of the drug loaded species of 6 or more. In one embodiment, the composition of the invention has 9% or less of the drug loaded species of 6 or more. In one embodiment, the composition of the invention has 8% or less of the drug loaded species of 6 or more. In one embodiment, the composition of the invention has 7% or less of the drug loaded species of 6 or more. In one embodiment, the composition of the invention has 6% or less of the drug loaded species of 6 or more. In one embodiment, the composition of the invention has 5% or less of the drug loaded species of 6 or more. In one embodiment, the composition of the invention has 4% or less of the drug loaded species of 6 or more. In further embodiments, the composition has 15% to 1% of the drug loaded species of 6 or more, 10% to 1% of the drug loaded species of 6 or more, 5% to 1% of the drug loaded species of 6 or more, 10% to 0.5% of the drug loaded species of 6 or more, or 5% to 0.5% of the drug loaded species of 6 or more.

In one embodiment, the methods of the invention may be used to produce a composition comprising ADCs with an average DAR of 4. Such a composition may be obtained by contacting an ADC mixture with an amount of hydrophobic resin in an species absorption process to form a resin mixture, wherein the ADC mixture comprises drug loaded species of 4 or less and drug loaded species of 6 or more, and wherein the amount of hydrophobic resin is 5 to 10 times the weight of the drug loaded species of 6 or more in the

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ADC mixture, and obtaining a supernatant from the resin mixture, such that the composition comprising ADCs with an average DAR of 4 or less is produced. In one embodiment, the composition of the invention comprises ADCs with an average DAR of 3.5 or less. In one embodiment of the invention, the composition comprises ADCs with an average DAR of 3 or less. In one embodiment, the composition of the invention comprises ADCs with an average DAR of 2-4. In one embodiment of the invention, the composition comprises ADCs with an average DAR of 2.4 – 3.6. In one embodiment, the composition comprises ADCs and has an average DAR of 4 or less, or, alternatively, an average DAR of 3.5 or less, an average DAR of 3 or less, or an average DAR of 2.5 or less.

In one embodiment, the methods of the invention may be used to produce a composition comprising ADCs with an average Drug-to-Antibody Ratio (DAR) of 4 or less and comprising less than 15% undesired ADCs. The method includes contacting an ADC mixture with a hydrophobic resin, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the undesired ADCs; and removing the hydrophobic resin from the ADC mixture, such that the composition with a mean DAR of 4 or less and comprising less than 15% undesired ADCs is produced. In one embodiment, the undesired ADCs are 6 and 8 drug loaded species. In one embodiment, the amount of hydrophobic resin added to the ADC mixture is a resin weight which is 5 to 10 times the weight of the undesired ADCs in the ADC mixture. In another embodiment, the amount of hydrophobic resin added to the ADC mixture is a resin weight which is 5 to 7 times the weight of the undesired ADCs in the ADC mixture. In one embodiment, the amount of hydrophobic resin added to the ADC mixture is a resin weight which is 3 to 12 times the weight of the undesired ADCs in the ADC mixture.

The DAR of an ADC may be measured according to common methods known in the art, including, but not limited to UV/VIS spectroscopic analysis of the ADC and analytical HIC and HPLC, e.g., HPLC-MS.

III. Antibody Drug Conjugates

The compositions and methods described herein are based, at least in part, on antibody drug conjugates (ADCs) comprising anti-EGFR antibodies, or antigen-binding portions thereof, that specifically bind to EGFR conjugated to auristatin.

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In particular, the present invention pertains to methods and compositions comprising an anti-EGFR antibody drug conjugate comprising an antibody or an antigen-binding portion thereof, that recognizes an EGFR epitope which is found in tumorigenic, hyperproliferative or abnormal cells, wherein the epitope is not detectable in normal or wild-type cells. Preferably, the antibody or antigen-binding portion thereof, does not bind to or recognize normal or wild-type cells containing normal or wild-type EGFR epitope in the absence of overexpression and in the presence of normal EGFR post-translational modification.

Anti-EGFR antibodies suitable for use in accordance with the present compositions and methods are typically monoclonal and can include, for example, chimeric (e.g., having a human constant region and mouse variable region), humanized, or human antibodies; single chain antibodies; or the like. The immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. For example, the anti-EGFR antibody used in the anti-EGFR antibody drug conjugate of the invention may be Antibody 1. The sequences and characteristics of antibody 1 are described below (see also WO 2011/041319 and US20110076232 (see, *e.g.,* antibody sequence of Figure 55), incorporated by reference in its entirety herein). Antibody 1 targets the over-expressed form of the epidermal growth factor receptor (EGFR) present in 50% of all cancers of epithelial origin.

In a particular embodiment of the present invention, the anti-EGFR antibody used in the anti-EGFR antibody drug conjugate of the invention recognizes amplified wild-type EGFR and the de2-7 EGFR. The anti-EGFR antibody of the invention demonstrates useful specificity, in that it recognizes de2-7 EGFR and amplified EGFR, but does not recognize normal, wild-type EGFR or the unique junctional peptide which is characteristic of de2-7 EGFR. Sequences for Antibody 1 are provided below.

As described above, Antibody 1 is a humanized anti-EGFR antibody. The heavy chain variable (VH) and constant (CH) regions of Antibody 1 are shown below as SEQ ID NOS: 1 and 5, respectively. The VH region CDR1, CDR2, and CDR3 (SEQ ID NOS: 2, 3, and 4, respectively) are indicated by underlining.

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Heavy Chain Variable Region amino acid sequence (SEQ ID NO: 1) (CDRs are underlined):

QVQLQESGPGLVKPSQTLSLTCTVSGYSISSDFAWNWIRQPPGKGLEWMGYISYSGNTR
CDR1 (SEQ ID NO: 2) CDR2 (SEQ ID NO: 3)
YQPSLKSRITISRDTSKNQFFLKLNSVTAADTATYYCVTAGRGFPYWGQGTLVTVSS
CDR3 (SEQ ID NO: 4)

Heavy Chain Constant Region amino acid sequence (SEQ ID NO: 5):

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP
ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS
FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

The light chain variable (VL) and constant (CL) regions of Antibody 1 are shown below as SEQ ID NOS: 6 and 10, respectively. The VL region CDR1, CDR2, and CDR3 (SEQ ID NOS: 7, 8, and 9, respectively) are indicated by underlining.

Light Chain Variable Region amino acid sequence (SEQ ID NO: 6) (CDRs are underlined)::

DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGWLQQKPGKSFKGLIYHGTNLDDGVPS
CDR1 (SEQ ID NO: 7) CDR2 (SEQ ID NO: 8)
RFSGSGSGTDYTLTISSLQPEDFATYYCVQYAQFPWTFGGGTKLEIKR
CDR3 (SEQ ID NO: 9)

Light Chain Constant Region amino acid sequence (SEQ ID NO: 10):

TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE
QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

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Thus, in one embodiment, the anti-EGFR antibody (used in the ADCs described herein) comprises a light chain variable region comprising a Complementarity Determining Region 1 (CDR1), CDR2, and CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9, respectively, and comprises a heavy chain variable region comprising a CDR1, CDR2, and CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4.

In one embodiment, the invention provides a formulation comprising an ADC comprising an anti-EGFR antibody (conjugated to an auristatin) having a light chain variable region comprising CDRs as described in the amino acid sequence of SEQ ID NO: 6, a heavy chain variable region comprising CDRs as described in the amino acid sequence of SEQ ID NO: 1.

In one embodiment, the anti-EGFR antibody (used in the ADCs described herein) comprises a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 6 and a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 1.

In a preferred embodiment, the ADC used in the methods and compositions of the invention comprises an anti-EGFR antibody, *e.g.*, Antibody 1, and an auristatin. In one embodiment, the auristatin is monomethylauristatin E (MMAE), *e.g.*, vc-MMAE. In one embodiment, the auristatin is or monomethylauristatin F (MMAF), *.e.g,* mc-MMAF.

Alternatively, other auristatin-based ADCs may be made in accordance with the methods of the invention, Examples of antibodies that may be used in making auristatin-ADCs include chimeric antibodies, human antibodies, and humanized antibodies.

Antibodies, including anti-EGFR antibodies, that may be used make ADCs, including anti-EGFR antibody drug conjugates, can be generated by any suitable method known in the art. For example, monoclonal antibodies can be prepared using a wide variety of techniques including, e.g., the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. Hybridoma techniques are generally discussed in, for example, Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed., 1988); and Hammerling, et al., In Monoclonal Antibodies and T-Cell Hybridomas, pp. 563-681 (Elsevier, N.Y., 1981). Examples of phage display methods that can be used to make the anti-CD70 antibodies include, e.g., those disclosed in Brinkman et al., 1995, J Immunol

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Methods 182:41-50; Ames et al., 1995, J Immunol Methods 184:177-186; Kettleborough et al., 1994, Eur J Immunol 24:952-958; Persic et al., 1997, Gene 187:9-18; Burton et al., 1994, Advances in Immunology 57:191-280; PCT Application No. PCT/GB91/01 134; PCT Publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Pat. Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108 (the disclosures of which are incorporated by reference herein).

Mammalian host cells for expressing the recombinant antibodies of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr-CHO cells, described in Urlaub and Chasin (1980) Proc. Natl. Acad. Sci. USA 77:4216-4220, used with a DHFR selectable marker, e.g., as described in Kaufman and Sharp (1982) J. Mol. Biol. 159:601-621) and DG44 or DUXB11 cells (Urlaub et al. (1986) Som. Cell Molec. Genet. 12:555; Haynes et al. (1983) Nuc. Acid. Res. 11:687-706; Lau et al. (1984) Mol. Cell. Biol. 4:1469-1475), NS0 myeloma cells, monkey kidney line (e.g., CVI and COS, such as a COS 7 cell), SP2 cells, human embryonic kidney (HEK) cells, such as a HEK-293 cell, Chinese hamster fibroblast (e.g., R1610), human cervical carcinoma (e.g., HELA), murine fibroblast (e.g., BALBc/3T3), murine myeloma (P3x63-Ag3.653; NS0; SP2/O), hamster kidney line (e.g., HAK), murine L cell (e.g., L-929), human lymphocyte (e.g., RAJI), human kidney (e.g., 293 and 293T). Host cell lines are typically commercially available (e.g., from BD Biosciences, Lexington, Ky.; Promega, Madison, Wis.; Life Technologies, Gaithersburg, Md.) or from the American Type Culture Collection (ATCC, Manassas, Va.).

When recombinant expression vectors encoding the antibody are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibodies in the host cells or secretion of the antibodies into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

In an exemplary system for recombinant expression of antibodies, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr-CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain cDNAs are each

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operatively linked to CMV enhancer/AdMLP promoter regulatory elements to drive high levels of transcription of the cDNAs. The recombinant expression vector also carries cDNA encoding DHFR, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium. Still further, the invention provides a method of synthesizing an antibody by culturing a host cell of the invention in a suitable culture medium until the antibody is synthesized. The method can further comprise isolating the antibody from the culture medium.

In a preferred embodiment, the anti-EGFR antibody, or an antigen-binding portion thereof, is conjugated to an auristatin (one or more). Auristatins have been shown to interfere with microtubule dynamics, GTP hydrolysis, and/or nuclear and cellular division and have anticancer and/or antifungal activity. Auristatins represent a group of dolastatin analogs that have generally been shown to possess anticancer activity by interfering with microtubule dynamics and GTP hydrolysis, thereby inhibiting cellular division. For example, Auristatin E (U.S. Patent No. 5,635,483, incorporated by reference herein) is a synthetic analogue of the marine natural product dolastatin 10, a compound that inhibits tubulin polymerization by binding to the same site on tubulin as the anticancer drug vincristine (G. R. Pettit, Prog. Chem. Org. Nat. Prod, 70: 1-79 (1997)). Dolastatin 10, auristatin PE, and auristatin E are linear peptides having four amino acids, three of which are unique to the dolastatin class of compounds. Exemplary embodiments of the auristatin subclass of mitotic inhibitors include, but are not limited to, monomethyl auristatin D (MMAD or auristatin D derivative), monomethyl auristatin E (MMAE or auristatin E derivative), monomethyl auristatin F (MMAF or an MMAF derivative), auristatin F phenylenediamine (AFP), auristatin EB (AEB), auristatin EFP (AEFP), and 5-benzoylvaleric acid-AE ester (AEVB). The synthesis and structure of auristatin derivatives are described in U.S. Patent Application Publication Nos. 2003-0083263, 2005-0238649 and 2005-0009751; International Patent Publication No. WO 04/010957, International Patent Publication No. WO 02/088172, and U.S. Pat. Nos. 6,323,315; 6,239,104; 6,034,065; 5,780,588; 5,665,860; 5,663,149; 5,635,483; 5,599,902;

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5,554,725; 5,530,097; 5,521,284; 5,504,191; 5,410,024; 5,138,036; 5,076,973; 4,986,988; 4,978,744; 4,879,278; 4,816,444; and 4,486,414, each of which is incorporated by reference herein.

In one embodiment, the anti-EGFR antibody of the invention is conjugated to at least one MMAF (monomethylauristatin F). Monomethyl auristatin F (MMAF) inhibits cell division by blocking the polymerization of tubulin. It has a charged C-terminal phenylalanine residue that attenuates its cytotoxic activity compared to its uncharged counterpart MMAE. Because of its super toxicity, it cannot be used as a drug itself, but can be linked to a monoclonal antibody (mAb) that directs it to the cancer cells. In one embodiment, the linker to the anti-EGFR antibody is stable in extracellular fluid, but is cleaved by cathepsin once the conjugate has entered a tumor cell, thus activating the anti-mitotic mechanism. In one embodiment, Antibody 1 is conjugated to MMAF using a noncleavable maleimidocaproyl (mc) linkage. The structure of MMAF is provided in Figure 1.

In one embodiment, the anti-EGFR antibody of the invention is conjugated to at least one MMAE (mono-methyl auristatin E). Monomethyl auristatin E (MMAE, vedotin) inhibits cell division by blocking the polymerisation of tubulin. Because of its super toxicity, it also cannot be used as a drug itself. In recent cancer therapy developments, it is linked to a monoclonal antibody (mAb) that recognizes a specific marker expression in cancer cells and directs MMAE to the cancer cells. In one embodiment, the linker linking MMAE to the anti-EGFR antibody is stable in extracellular fluid (i.e., the medium or environment that is external to cells), but is cleaved by cathepsin once the ADC has bound to the specific cancer cell antigen and entered the cancer cell, thus releasing the toxic MMAE and activating the potent anti-mitotic mechanism. The structure of MMAE is provided in Figure 1.

Techniques for conjugating therapeutic agents to proteins, and in particular to antibodies, are well-known. (See, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy," in Monoclonal Antibodies And Cancer Therapy (Reisfeld et al. eds., Alan R. Liss, Inc., 1985); Hellstrom et al., "Antibodies For Drug Delivery," in Controlled Drug Delivery (Robinson et al. eds., Marcel Dekker, Inc., 2nd ed. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," in Monoclonal Antibodies '84: Biological And Clinical Applications (Pinchera et al. eds., 1985); "Analysis, Results, and Future Prospective of the Therapeutic Use of Radiolabeled

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Antibody In Cancer Therapy," in Monoclonal Antibodies For Cancer Detection And Therapy (Baldwin et al. eds., Academic Press, 1985); and Thorpe et al., 1982, Immunol. Rev. 62:119-58. See also, e.g., PCT publication WO 89/12624.)

In one embodiment, the anti-EGFR-ADC comprises a linker region between the cytotoxic drug and the antibody. For example, such linker, spacer and/or stretcher compounds include, but are not limited to, the following: amino benzoic acid spacers (see, for example and without limitation, U.S. Patent Nos. 7,091,186 and 7,553,816, each of which is hereby incorporated by reference in its entirety); maleimidocaproyl; p-aminobenzylcarbamoyl (PAB); lysosomal enzyme-cleavable linkers (see, for example and without limitation, U.S. Patent No. 6,214,345, hereby incorporated by reference in its entirety); maleimidocaproyl-polyethylene 20 glycol (MC(PEG)6-OH); N-methyl-valine citrulline; N-succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) (see, for example and without limitation, Yoshitake et al. (1979) Eur. J. Biochem., 101, 395-399, hereby incorporated by reference in its entirety); N- succinimidyl 4-(2-pyridyldithio) butanoate (SPDB) (see, for example and without limitation, U.S. Patent No. 4,563,304, hereby incorporated by reference 25 in its entirety); N-Succinimidyl 4-(2-pyridylthio)pentanoate (SPP); valine-citrulline (vc); and other linker, spacer, and/or stretcher compounds (see, for example and without limitation, U.S. Patent Nos. 7,090,843, 7,223,837, and 7,659,241, and U.S. Patent Publication Nos. 2004/0018194, 2004/0121940, 2006/0116422, 2007/0258987, 2008/0213289, 2008/0241128, 2008/0311136, 2008/0317747, and 2009/0010945, each of which is hereby incorporated by reference in its entirety). Generally speaking, techniques for attaching and/or conjugating the agents set forth above, as well as other agents, to specific binding members of the present invention, particularly antibodies and fragments thereof, are known in the art. See, for example and without limitation, Amon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", In Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of

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Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982), each of which is hereby incorporated by reference in its entirety.

A number of different reactions are available for covalent attachment of drugs to antibodies. This is often accomplished by reaction of the amino acid residues of the antibody molecule, including the amine groups of lysine, the free carboxylic acid groups of glutamic and aspartic acid, the sulfhydryl groups of cysteine and the various moieties of the aromatic amino acids. One of the most commonly used non-specific methods of covalent attachment is the carbodiimide reaction to link a carboxy (or amino) group of a compound to amino (or carboxy) groups of the antibody. Additionally, bifunctional agents such as dialdehydes or imidoesters have been used to link the amino group of a compound to amino groups of the antibody molecule. Also available for attachment of drugs to antibodies is the Schiff base reaction. This method involves the periodate oxidation of a drug that contains glycol or hydroxy groups, thus forming an aldehyde which is then reacted with the antibody molecule. Attachment occurs via formation of a Schiff base with amino groups of the antibody molecule. Isothiocyanates can also be used as coupling agents for covalently attaching drugs to antibodies. Other techniques are known to the skilled artisan and within the scope of the present invention. Non-limiting examples of such techniques are described in, e.g., U.S. Pat. Nos. 5,665,358; 5,643,573; and 5,556,623, which are incorporated by reference in their entireties herein.

In certain embodiments, an intermediate, which is the precursor of the linker, is reacted with the drug under appropriate conditions. In certain embodiments, reactive groups are used on the drug and/or the intermediate. The product of the reaction between the drug and the intermediate, or the derivatized drug, is subsequently reacted with the anti-EGFR antibody under appropriate conditions.

Other examples of conjugation methods are described in US Patent No. 7,837,980 (Seattle Genetics), Carter and Senter (2008) *Cancer J*, 14(3):154, as well as U.S. Published Application Nos. 2004-0157782 A1 and 2005-0238649 and International Patent Application No, PCT/US04/038392.

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In one embodiment of the invention, the anti-EGFR antibody, or antigen-binding portion thereof, is conjugated to an auristatin which is MMAF. In one embodiment, the anti-EGFR ADC is Antibody 1-mc-MMAF. Antibody 1-mc-MMAF comprises antibody 1 (described above and in SEQ ID NOs: 1 to 10) covalently linked to one or more molecules of monomethyl auristatin F (MMAF) (see Figure 1 for structure). To generate Antibody 1-mc-MMAF, the interchain disulfide bonds of antibody 1 are reduced to sulfhydryl groups. MMAF is then coupled to the antibody via these sulfhydryl groups. Antibody 1-mc-MMAF is generated using a noncleavable linker, *i.e.*, a noncleavable maleimidocaproyl (mc) linkage, as shown in Figure 1.

In one embodiment of the invention, the anti-EGFR antibody, or antigen-binding portion thereof, is conjugated to an auristatin which is MMAE. In one embodiment, the anti-EGFR ADC comprises Antibody 1-vc-MMAE. Antibody 1-vc-MMAE comprises Antibody 1 (described above and in SEQ ID NOs: 1 to 10) covalently linked to one or more molecules of monomethyl auristatin E (MMAE) (see Figure 1 for structure). To generate Antibody 1-vc-MMAE, the interchain disulfide bonds of Antibody 1 are reduced to sulfhydryl groups. vcMMAE is then coupled to the antibody via these sulfhydryl groups. Antibody 1-vc-MMAE is generated using a valine citrulline linker (vc), as shown in Figure 1, thus forming Antibody 1-vc-MMAE

IV. Composition Uses

In accordance with the present methods, a composition comprising anti-EGFR ADCs having a desired average DAR is administered to a subject having (or at risk of having) a disorder requiring treatment with the anti-EGFR antibody. The formulation comprising the anti-EGFR ADC may be administered either alone or in combination with other compositions in the prevention or treatment of the disorder requiring treatment with the anti-EGFR antibody.

As used herein, the term "a disorder in which EGFR activity is detrimental" is intended to include diseases and other disorders in which the presence of EGFR in a subject suffering from the disorder has been shown to be or is suspected of being either responsible for the pathophysiology of the disorder or a factor that contributes to a worsening of the disorder. Accordingly, a disorder in which EGFR activity is detrimental is a disorder in which

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inhibition of EGFR activity is expected to alleviate the symptoms and/or progression of the disorder. Such disorders may be evidenced, for example, by an increase in the activity of EGFR or an increase in the amount of EGFR present in a biological sample from a subject suffering from the disorder (e.g., an increase in the concentration of EGFR in a tissue sample, in serum, plasma, synovial fluid, etc. of the subject), which can be detected, for example, using an anti-EGFR antibody.

Thus, the ADC compositions of the invention having an average DAR of, for example, 2-4, may be used to treat cancer. Examples of cancer that may be treated include, but are not limited to, glioblastoma, non-small cell lung cancer, squamous non-small cell lung cancer (NSCLC), lung cancer, colon cancer, head and neck cancer, breast cancer, squamous cell tumors, anal cancer, skin cancer, and vulvar cancer. ADC compositions having a desired average DAR may also be used to treat a subject having a solid tumor likely to over-express the Epidermal Growth Factor Receptor (EGFR) or glioblastoma multiforme.

In one embodiment, the purified ADC compositions of the invention having an average DAR of, for example, 2-4, are used to treat colorectal cancer, head and neck cancer (including, but not limited to, hypopharyngeal cancer, oropharyngeal cancer, esophageal cancer, laryngeal cancer, and oral cavity cancer), non-small cell lung cancer, squamous non-small cell lung cancer (NSCLC), pancreatic cancer, gastric cancer, solid tumors, a solid tumor likely to over-express the Epidermal Growth Factor Receptor (EGFR), glioblastoma multiforme, and breast cancer. More particular examples of such cancers include squamous tumors (including, squamous tumors of the lung, head and neck, cervical, etc.), glioblastoma, glioma, lung cancer, colon cancer, head and neck cancer, breast cancer, squamous cell tumors, anal cancer, skin cancer, and vulvar cancer.

The unique specificity of the compositions comprising anti-EGFR ADCs provides diagnostic and therapeutic uses to identify, characterize, target and treat, reduce or eliminate a number of tumorigenic cell types and tumor types, for example, but not limited to, glioblastoma, non-small cell lung cancer, lung cancer, colon cancer, head and neck cancer, breast cancer, squamous cell tumors, anal cancer, skin cancer, a solid tumor likely to over-express the Epidermal Growth Factor Receptor (EGFR), glioblastoma multiforme, and vulvar cancer, without the problems associated with normal tissue uptake that may be seen with previously known EGFR antibodies. Thus, cells overexpressing EGFR (e.g. by amplification

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or expression of a mutant or variant EGFR), and in particular embodiments, those demonstrating aberrant post-translational modification may be recognized, isolated, characterized, targeted and treated or eliminated utilizing the antibody(ies) or fragments thereof of the present invention. The compositions of the invention may be used to treat EGFR positive tumors. Methods for detecting expression of EGFR in a tumor are known in the art, e.g., the EGFR pharmDx™ Kit (Dako). In contrast, an "EGFR negative tumor" is defined as a tumor having an absence of EGFR membrane staining above background in a tumor sample as determined by immunohistochemical techniques.

In one aspect of the invention, there is provided a method for treating a subject comprising administering a therapeutically effective amount of an anti-EGFR ADC in any of the compositions as described herein, wherein the subject has a disorder requiring treatment with the anti-EGFR antibody in the composition (*e.g.* a tumor, a cancerous condition, a precancerous condition, and any condition related to or resulting from hyperproliferative cell growth).

A composition comprising anti-EGFR ADCs can thus specifically categorize the nature of EGFR tumors or tumorigenic cells, by staining or otherwise recognizing those tumors or cells wherein EGFR overexpression, particularly amplification and/or EGFR mutation, particularly de2-7EGFR, is present.

Therefore, in a further aspect of the invention, there is provided a method of treatment of a tumor, a cancerous condition, a precancerous condition, and any condition related to or resulting from hyperproliferative cell growth comprising administration of a composition of the invention comprising an anti-EGFR ADC comprising Antibody 1.

Various delivery systems are known and can be used to administer the anti-EGFR ADC composition of the invention. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The ADCs can be administered, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, and the like) and can be administered together with other biologically active agents such as chemotherapeutic agents. Administration can be systemic or local. In one embodiment, the formulation of the invention is delivered to a subject intravenously. In another embodiment, the formulation of the invention is delivered to a subject

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subcutaneously. In one embodiment, the subject administers the formulation to himself/herself (self-administration).

The amount of the ADC that is effective in the treatment or prevention of a disorder requiring treatment with the anti-EGFR antibody in the formulation, e.g. a cancer, can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the stage of immunological disorder or EGFR-expressing cancer, and should be decided according to the judgment of the practitioner and each patient's circumstances. In one embodiment, a therapeutically effective amount of the formulation is administered. The term "therapeutically effective amount" or "effective amount" of an antibody as used herein refers to an amount effective in the prevention or treatment or alleviation of a symptom of a disorder for the treatment of which the antibody is effective. An example of a therapeutically effective amount of the formulation is an amount sufficient to inhibit detrimental EGFR activity or treat a disorder in which EGFR activity is detrimental.

A dose of an anti-EGFR ADC can be administered, for example, daily, once per week (weekly), twice per week, thrice per week, four times per week, five times per week, biweekly, every three weeks, monthly, every four weeks, two weeks on / one week off or otherwise as needed.

Thus, pharmaceutical compositions according to the present invention, and for use in accordance with the present invention, may comprise, in addition to the active ingredient (ADC), a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material may depend on the route of administration, which may be oral, or by injection, e.g. intravenous. In one embodiment, the pharmaceutical composition comprises an ADC (e.g., an anti-EGFR antibody such as Antibody 1 conjugated to a MMAE or MMAF), and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline,

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phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition.

In certain embodiments, the anti-EGFR ADC can be co-administered to a subject with one or more additional therapeutic agents to treat cancer. The term "co-administered" means the administration of two or more different pharmaceutical agents or treatments (e.g., radiation treatment) that are administered to a subject by combination in the same pharmaceutical composition or separate pharmaceutical compositions. Thus co-administration involves administration at the same time of a single pharmaceutical composition comprising two or more pharmaceutical agents or administration of two or more different compositions to the same subject at the same or different times.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, where examples of the agents include, such as radiation, alkylating agents, angiogenesis inhibitors, antibodies, antimetabolites, antimitotics, antiproliferatives, antivirals, aurora kinase inhibitors, apoptosis promoters (for example, Bcl-xL, Bcl-w and Bfl-1) inhibitors, activators of death receptor pathway, Bcr-Abl kinase inhibitors, BiTE (Bi-Specific T cell Engager) antibodies, antibody drug conjugates, biologic response modifiers, cyclin-dependent kinase inhibitors, cell cycle inhibitors, cyclooxygenase-2 inhibitors, DVDs (dual variable domain antibodies), leukemia viral oncogene homolog (ErbB2) receptor inhibitors, growth factor inhibitors, heat shock protein (HSP)-90 inhibitors, histone deacetylase (HDAC) inhibitors, hormonal therapies, immunologicals, inhibitors of inhibitors of apoptosis proteins (IAPs), intercalating antibiotics, kinase inhibitors, kinesin inhibitors, Jak2 inhibitors, mammalian target of rapamycin inhibitors, microRNA's, mitogen-activated extracellular signal-regulated kinase inhibitors, multivalent binding proteins, non-steroidal anti-inflammatory drugs (NSAIDs), poly ADP (adenosine diphosphate)-ribose polymerase (PARP) inhibitors, platinum chemotherapeutics, polo-like kinase (Plk) inhibitors, phosphoinositide-3 kinase (bromodomain) inhibitors, proteosome inhibitors, purine analogs, pyrimidine analogs, receptor tyrosine kinase inhibitors, etinoids/deltoids plant alkaloids, small inhibitory ribonucleic acids (siRNAs), topoisomerase inhibitors, temozolomide, ubiquitin ligase inhibitors, and the like, and in combination with one or more of these agents .

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Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including BiTE antibodies, which are bi-specific antibodies that direct T-cells to attack cancer cells by simultaneously binding the two cells. The T-cell then attacks the target cancer cell. Examples of BiTE antibodies include adecatumumab (Micromet MT201), blinatumomab (Micromet MT103) and the like. Without being limited by theory, one of the mechanisms by which T-cells elicit apoptosis of the target cancer cell is by exocytosis of cytolytic granule components, which include perforin and granzyme B. In this regard, Bcl-2 has been shown to attenuate the induction of apoptosis by both perforin and granzyme B. These data suggest that inhibition of Bcl-2 could enhance the cytotoxic effects elicited by T-cells when targeted to cancer cells (V.R. Sutton, D.L. Vaux and J.A. Trapani, *J. of Immunology* 1997, 158 (12), 5783).

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including siRNA. SiRNAs are molecules having endogenous RNA bases or chemically modified nucleotides. The modifications do not abolish cellular activity, but rather impart increased stability and/or increased cellular potency. Examples of chemical modifications include phosphorothioate groups, 2'-deoxynucleotide, 2'-OCH_3-containing ribonucleotides, 2'-F-ribonucleotides, 2'-methoxyethyl ribonucleotides, combinations thereof and the like. The siRNA can have varying lengths (e.g., 10-200 bps) and structures (e.g., hairpins, single/double strands, bulges, nicks/gaps, mismatches) and are processed in cells to provide active gene silencing. A double-stranded siRNA (dsRNA) can have the same number of nucleotides on each strand (blunt ends) or asymmetric ends (overhangs). The overhang of 1-2 nucleotides can be present on the sense and/or the antisense strand, as well as present on the 5'- and/ or the 3'-ends of a given strand.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including DVDs and other multivalent binding proteins. Multivalent binding proteins are binding proteins comprising two or more antigen binding sites. Multivalent binding proteins are engineered to have the three or more antigen binding sites and are generally not naturally occurring antibodies. The term "multispecific binding protein" means a binding protein capable of binding two or more related or unrelated targets. Dual variable domain (DVD) binding proteins are tetravalent or multivalent binding proteins binding proteins comprising two or more antigen binding sites. Such DVDs may be

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monospecific (i.e., capable of binding one antigen) or multispecific (i.e., capable of binding two or more antigens). DVD binding proteins comprising two heavy chain DVD polypeptides and two light chain DVD polypeptides are referred to as DVD Ig's. Each half of a DVD Ig comprises a heavy chain DVD polypeptide, a light chain DVD polypeptide, and two antigen binding sites. Each binding site comprises a heavy chain variable domain and a light chain variable domain with a total of 6 CDRs involved in antigen binding per antigen binding site. Multispecific DVDs include DVD binding proteins that bind DLL4 and VEGF, or C-met and EGFR or ErbB3 and EGFR.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including alkylating agents. Alkylating agents include altretamine, AMD-473, AP-5280, apaziquone, bendamustine, brostallicin, busulfan, carboquone, carmustine (BCNU), chlorambucil, CLORETAZINE® (laromustine, VNP 40101M), cyclophosphamide, decarbazine, estramustine, fotemustine, glufosfamide, ifosfamide, KW-2170, lomustine (CCNU), mafosfamide, melphalan, mitobronitol, mitolactol, nimustine, nitrogen mustard N-oxide, ranimustine, temozolomide, thiotepa, TREANDA® (bendamustine), treosulfan, rofosfamide and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including angiogenesis inhibitors. Angiogenesis inhibitors include endothelial-specific receptor tyrosine kinase (Tie-2) inhibitors, epidermal growth factor receptor (EGFR) inhibitors, insulin growth factor-2 receptor (IGFR-2) inhibitors, matrix metalloproteinase-2 (MMP-2) inhibitors, matrix metalloproteinase-9 (MMP-9) inhibitors, platelet-derived growth factor receptor (PDGFR) inhibitors, thrombospondin analogs, vascular endothelial growth factor receptor tyrosine kinase (VEGFR) inhibitors and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including antimetabolites. Antimetabolites include ALIMTA® (pemetrexed disodium, LY231514, MTA), 5-azacitidine, XELODA® (capecitabine), carmofur, LEUSTAT® (cladribine), clofarabine, cytarabine, cytarabine ocfosfate, cytosine arabinoside, decitabine, deferoxamine, doxifluridine, eflornithine, EICAR (5-ethynyl-1-β -D-ribofuranosylimidazole-4-carboxamide), enocitabine, ethnylcytidine,

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fludarabine, 5-fluorouracil alone or in combination with leucovorin, GEMZAR® (gemcitabine), hydroxyurea, ALKERAN®(melphalan), mercaptopurine, 6-mercaptopurine riboside, methotrexate, mycophenolic acid, nelarabine, nolatrexed, ocfosfate, pelitrexol, pentostatin, raltitrexed, Ribavirin, triapine, trimetrexate, S-1, tiazofurin, tegafur, TS-1, vidarabine, UFT and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including antivirals. Antivirals include ritonavir, hydroxychloroquine and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including aurora kinase inhibitors. Aurora kinase inhibitors include ABT-348, AZD-1152, MLN-8054, VX-680, Aurora A-specific kinase inhibitors, Aurora B-specific kinase inhibitors and pan-Aurora kinase inhibitors and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including Bcl-2 protein inhibitors. Bcl-2 protein inhibitors include AT-101 ((-)gossypol), GENASENSE® (G3139 or oblimersen (Bcl-2-targeting antisense oligonucleotide)), IPI-194, IPI-565, N-(4-(4-((4'-chloro(1,1'-biphenyl)-2-yl)methyl)piperazin-1-yl)benzoyl)-4-(((1R)-3-(dimethylamino)-1-((phenylsulfanyl)methyl)propyl)amino)-3-nitrobenzenesulfonamide) (ABT-737), N-(4-(4-((2-(4-chlorophenyl)-5,5-dimethyl-1-cyclohex-1-en-1-yl)methyl)piperazin-1-yl)benzoyl)-4-(((1R)-3-(morpholin-4-yl)-1-((phenylsulfanyl)methyl)propyl)amino)-3-((trifluoromethyl)sulfonyl)benzenesulfonamide (ABT-263), GX-070 (obatoclax), ABT-199, and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including Bcr-Abl kinase inhibitors, such as DASATINIB® (BMS-354825), GLEEVEC® (imatinib) and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including CDK inhibitors. CDK inhibitors include AZD-5438, BMI-1040, BMS-032, BMS-387, CVT-2584, flavopyridol, GPC-286199, MCS-5A, PD0332991, PHA-690509, seliciclib (CYC-202, R-roscovitine), ZK-304709 and the like.

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Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including COX-2 inhibitors. COX-2 inhibitors include ABT-963, ARCOXIA® (etoricoxib), BEXTRA® (valdecoxib), BMS347070, CELEBREX® (celecoxib), COX-189 (lumiracoxib), CT-3, DERAMAXX® (deracoxib), JTE-522, 4-methyl-2-(3,4-dimethylphenyl)-1-(4-sulfamoylphenyl-1H-pyrrole), MK-663 (etoricoxib), NS-398, parecoxib, RS-57067, SC-58125, SD-8381, SVT-2016, S-2474, T-614, VIOXX® (rofecoxib) and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including other EGFR inhibitors. EGFR inhibitors include EGFR antibodies, ABX-EGF, anti-EGFR immunoliposomes, EGF-vaccine, EMD-7200, ERBITUX® (cetuximab), HR3, IgA antibodies, IRESSA® (gefitinib), TARCEVA® (erlotinib or OSI-774), TP-38, EGFR fusion protein, TYKERB® (lapatinib) and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including HER2 inhibitors. ErbB2 receptor inhibitors include CP-724-714, CI-1033 (canertinib), HERCEPTIN® (trastuzumab), TYKERB® (lapatinib), OMNITARG® (2C4, petuzumab), TAK-165, GW-572016 (ionafarnib), GW-282974, EKB-569, PI-166, dHER2 (HER2 vaccine), APC-8024 (HER-2 vaccine), anti-HER/2neu bispecific antibody, B7.her2IgG3, AS HER2 trifunctional bispecfic antibodies, mAB AR-209, mAB 2B-1 and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including histone deacetylase inhibitors, such as depsipeptide, LAQ-824, MS-275, trapoxin, suberoylanilide hydroxamic acid (SAHA), TSA, valproic acid and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including HSP-90 inhibitors include 17-AAG-nab, 17-AAG, CNF-101, CNF-1010, CNF-2024, 17-DMAG, geldanamycin, IPI-504, KOS-953, MYCOGRAB® (human recombinant antibody to HSP-90), NCS-683664, PU24FCl, PU-3, radicicol, SNX-2112, STA-9090 VER49009 and the like.

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Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including inhibitors of inhibitors of apoptosis proteins, such as HGS1029, GDC-0145, GDC-0152, LCL-161, LBW-242 and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including other ADCs, such as anti-CD22-MC-MMAF, anti-CD22-MC-MMAE, anti-CD22-MCC-DM1, CR-011-vcMMAE, PSMA-ADC, MEDI-547, SGN-19Am SGN-35, SGN-75 and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including activators of death receptor pathway, such as TRAIL, antibodies or other agents that target TRAIL or death receptors (e.g., DR4 and DR5) such as Apomab, conatumumab, ETR2-ST01, GDC0145, (lexatumumab), HGS-1029, LBY-135, PRO-1762 and trastuzumab.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including kinesin inhibitors, such as Eg5 inhibitors such as AZD4877, ARRY-520; CENPE inhibitors such as GSK923295A and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including JAK-2 inhibitors, such as CEP-701 (lesaurtinib), XL019 and INCB018424 and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including MEK inhibitors, such as ARRY-142886, ARRY-438162 PD-325901, PD-98059 and the like.

Anti-EGFR ADCs (or formulations comprising anti-EGFR ADCs) can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including mTOR inhibitors, such as AP-23573, CCI-779, everolimus, RAD-001, rapamycin, temsirolimus, ATP-competitive TORC1/TORC2 inhibitors, including PI-103, PP242, PP30, Torin 1 and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including non-steroidal anti-inflammatory drugs (NSAIDs), such as AMIGESIC® (salsalate), DOLOBID® (diflunisal), MOTRIN®

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(ibuprofen), ORUDIS® (ketoprofen), RELAFEN® (nabumetone), FELDENE® (piroxicam), ibuprofen cream, ALEVE® (naproxen) and NAPROSYN® (naproxen), VOLTAREN® (diclofenac), INDOCIN® (indomethacin), CLINORIL® (sulindac), TOLECTIN® (tolmetin), LODINE® (etodolac), TORADOL® (ketorolac), DAYPRO® (oxaprozin) and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including PDGFR inhibitors, such as C-451, CP-673, CP-868596 and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including platinum chemotherapeutics, such as cisplatin, ELOXATIN® (oxaliplatin) eptaplatin, lobaplatin, nedaplatin, PARAPLATIN® (carboplatin), satraplatin, picoplatin and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including polo-like kinase inhibitors, *e.g.*, BI-2536 and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including phosphoinositide-3 kinase (PI3K) inhibitors, such as wortmannin, LY294002, XL-147, CAL-120, ONC-21, AEZS-127, ETP-45658, PX-866, GDC-0941, BGT226, BEZ235, XL765 and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including thrombospondin analogs, such as ABT-510 (thrombospondin mimetic), ABT-567, ABT-898 (thrombospondin-1 mimetic peptide), TSP-1 and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including VEGFR inhibitors, such as AVASTIN® (bevacizumab), ABT-869, AEE-788, ANGIOZYME™ (a ribozyme that inhibits angiogenesis (Ribozyme Pharmaceuticals (Boulder, CO.) and Chiron, (Emeryville, CA)), axitinib (AG-13736), AZD-2171, CP-547,632, IM-862, MACUGEN (pegaptamib), NEXAVAR® (sorafenib, BAY43-9006), pazopanib (GW-786034), vatalanib (PTK-787, ZK-222584), SUTENT® (sunitinib, SU-11248), VEGF trap, ZACTIMA™ (vandetanib, ZD-6474), GA101,

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ofatumumab, ABT-806 (mAb-806), ErbB3 specific antibodies, BSG2 specific antibodies, DLL4 specific antibodies and C-met specific antibodies, and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including antibiotics, such as intercalating antibiotics aclarubicin, actinomycin D, amrubicin, annamycin, adriamycin, BLENOXANE® (bleomycin), daunorubicin, CAELYX® or MYOCET® (liposomal doxorubicin), elsamitrucin, epirbucin, glarbuicin, ZAVEDOS® (idarubicin), mitomycin C, nemorubicin, neocarzinostatin, peplomycin, pirarubicin, rebeccamycin, stimalamer, streptozocin, VALSTAR® (valrubicin), zinostatin and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including topoisomerase inhibitors, such as aclarubicin, 9-aminocamptothecin, amonafide, amsacrine, becatecarin, belotecan, BN-80915, CAMPTOSAR® (irinotecan hydrochloride), camptothecin, CARDIOXANE® (dexrazoxine), diflomotecan, edotecarin, ELLENCE® or PHARMORUBICIN® (epirubicin), etoposide, exatecan, 10-hydroxycamptothecin, gimatecan, lurtotecan, mitoxantrone, orathecin, pirarbucin, pixantrone, rubitecan, sobuzoxane, SN-38, tafluposide, topotecan and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including therapeutic antibodies, such as AVASTIN® (bevacizumab), CD40-specific antibodies, chTNT-1/B, denosumab, ERBITUX® (cetuximab), HUMAX-CD4® (zanolimumab), IGF1R-specific antibodies, lintuzumab, PANOREX® (edrecolomab), RENCAREX® (WX G250), RITUXAN® (rituximab), ticilimumab, trastuzimab, CD20 antibodies types I and II and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including hormonal therapies, such as ARIMIDEX® (anastrozole), AROMASIN® (exemestane), arzoxifene, CASODEX® (bicalutamide), CETROTIDE® (cetrorelix), degarelix, deslorelin, DESOPAN® (trilostane), dexamethasone, DROGENIL® (flutamide), EVISTA® (raloxifene), AFEMA™ (fadrozole), FARESTON® (toremifene), FASLODEX® (fulvestrant), FEMARA® (letrozole), formestane, glucocorticoids, HECTOROL® (doxercalciferol), RENAGEL® (sevelamer carbonate), lasofoxifene, leuprolide acetate, MEGACE® (megesterol), MIFEPREX® (mifepristone),

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NILANDRON™ (nilutamide), NOLVADEX® (tamoxifen citrate), PLENAXIS™ (abarelix), prednisone, PROPECIA® (finasteride), rilostane, SUPREFACT® (buserelin), TRELSTAR® (luteinizing hormone releasing hormone (LHRH)), VANTAS® (Histrelin implant), VETORYL® (trilostane or modrastane), ZOLADEX® (fosrelin, goserelin) and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including deltoids and retinoids, such as seocalcitol (EB1089, CB1093), lexacalcitrol (KH1060), fenretinide, PANRETIN® (aliretinoin), ATRAGEN® (liposomal tretinoin), TARGRETIN® (bexarotene), LGD-1550 and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including PARP inhibitors, such as ABT-888 (veliparib), olaparib, KU-59436, AZD-2281, AG-014699, BSI-201, BGP-15, INO-1001, ONO-2231 and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including plant alkaloids, such as, but are not limited to, vincristine, vinblastine, vindesine, vinorelbine and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including proteasome inhibitors, such as VELCADE® (bortezomib), MG132, NPI-0052, PR-171 and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including immunologicals. Examples of immunologicals include interferons and other immune-enhancing agents. Interferons include interferon alpha, interferon alpha-2a, interferon alpha-2b, interferon beta, interferon gamma-1a, ACTIMMUNE® (interferon gamma-1b) or interferon gamma-n1, combinations thereof and the like. Other agents include ALFAFERONE®,(IFN-α), BAM-002 (oxidized glutathione), BEROMUN® (tasonermin), BEXXAR® (tositumomab), CAMPATH® (alemtuzumab), CTLA4 (cytotoxic lymphocyte antigen 4), decarbazine, denileukin, epratuzumab, GRANOCYTE® (lenograstim), lentinan, leukocyte alpha interferon, imiquimod, MDX-010 (anti-CTLA-4), melanoma vaccine, mitumomab, molgramostim, MYLOTARG™ (gemtuzumab ozogamicin), NEUPOGEN® (filgrastim), OncoVAC-CL, OVAREX® (oregovomab), pemtumomab (Y-muHMFG1), PROVENGE® (sipuleucel-T),

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sargaramostim, sizofilan, teceleukin, THERACYS® (Bacillus Calmette-Guerin), ubenimex, VIRULIZIN® (immunotherapeutic, Lorus Pharmaceuticals), Z-100 (Specific Substance of Maruyama (SSM)), WF-10 (Tetrachlorodecaoxide (TCDO)), PROLEUKIN® (aldesleukin), ZADAXIN® (thymalfasin), ZENAPAX® (daclizumab), ZEVALIN® (90Y-Ibritumomab tiuxetan) and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including biological response modifiers, such as agents that modify defense mechanisms of living organisms or biological responses, such as survival, growth or differentiation of tissue cells to direct them to have anti-tumor activity and include krestin, lentinan, sizofiran, picibanil PF-3512676 (CpG-8954), ubenimex and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including pyrimidine analogs, such as cytarabine (ara C or Arabinoside C), cytosine arabinoside, doxifluridine, FLUDARA® (fludarabine), 5-FU (5-fluorouracil), floxuridine, GEMZAR® (gemcitabine), TOMUDEX® (ratitrexed), TROXATYL™ (triacetyluridine troxacitabine) and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including purine analogs, such as LANVIS® (thioguanine) and PURI-NETHOL® (mercaptopurine).

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including antimitotic agents, such as batabulin, epothilone D (KOS-862), N-(2-((4-hydroxyphenyl)amino)pyridin-3-yl)-4-methoxybenzenesulfonamide, ixabepilone (BMS 247550), paclitaxel, TAXOTERE® (docetaxel), PNU100940 (109881), patupilone, XRP-9881 (larotaxel), vinflunine, ZK-EPO (synthetic epothilone) and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including ubiquitin ligase inhibitors, such as MDM2 inhibitors, such as nutlins, NEDD8 inhibitors such as MLN4924 and the like.

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Compounds of this invention can also be used as radiosensitizers that enhance the efficacy of radiotherapy. Examples of radiotherapy include external beam radiotherapy, teletherapy, brachytherapy and sealed, unsealed source radiotherapy and the like.

Anti-EGFR ADCs can be co-administered with a therapeutically effective amount of one or more agents to treat a cancer, including chemotherapeutic agents such as ABRAXANE™ (ABI-007), ABT-100 (farnesyl transferase inhibitor), ADVEXIN® (Ad5CMV-p53 vaccine), ALTOCOR® or MEVACOR® (lovastatin), AMPLIGEN® (poly I:poly C12U, a synthetic RNA), APTOSYN® (exisulind), AREDIA® (pamidronic acid), arglabin, L-asparaginase, atamestane (1-methyl-3,17-dione-androsta-1,4-diene), AVAGE® (tazarotene), AVE-8062 (combreastatin derivative) BEC2 (mitumomab), cachectin or cachexin (tumor necrosis factor), canvaxin (vaccine), CEAVAC® (cancer vaccine), CELEUK® (celmoleukin), CEPLENE® (histamine dihydrochloride), CERVARIX® (human papillomavirus vaccine), CHOP® (C: CYTOXAN® (cyclophosphamide); H: ADRIAMYCIN® (hydroxydoxorubicin); O: Vincristine (ONCOVIN®); P: prednisone), CYPAT™ (cyproterone acetate), combrestatin A4P, DAB(389)EGF (catalytic and translocation domains of diphtheria toxin fused via a His-Ala linker to human epidermal growth factor) or TransMID-107R™ (diphtheria toxins), dacarbazine, dactinomycin, 5,6-dimethylxanthenone-4-acetic acid (DMXAA), eniluracil, EVIZON™ (squalamine lactate), DIMERICINE® (T4N5 liposome lotion), discodermolide, DX-8951f (exatecan mesylate), enzastaurin, EPO906 (epithilone B), GARDASIL® (quadrivalent human papillomavirus (Types 6, 11, 16, 18) recombinant vaccine), GASTRIMMUNE®, GENASENSE®, GMK (ganglioside conjugate vaccine), GVAX® (prostate cancer vaccine), halofuginone, histerelin, hydroxycarbamide, ibandronic acid, IGN-101, IL-13-PE38, IL-13-PE38QQR (cintredekin besudotox), IL-13-pseudomonas exotoxin, interferon-α, interferon-γ, JUNOVAN™ or MEPACT™ (mifamurtide), lonafarnib, 5,10-methylenetetrahydrofolate, miltefosine (hexadecylphosphocholine), NEOVASTAT®(AE-941), NEUTREXIN® (trimetrexate glucuronate), NIPENT® (pentostatin), ONCONASE® (a ribonuclease enzyme), ONCOPHAGE® (melanoma vaccine treatment), ONCOVAX® (IL-2 Vaccine), ORATHECIN™ (rubitecan), OSIDEM® (antibody-based cell drug), OVAREX® MAb (murine monoclonal antibody), paclitaxel, PANDIMEX™ (aglycone saponins from ginseng comprising 20(S)protopanaxadiol (aPPD) and 20(S)protopanaxatriol (aPPT)), panitumumab,

PANVAC®-VF (investigational cancer vaccine), pegaspargase, PEG Interferon A, phenoxodiol, procarbazine, rebimastat, REMOVAB® (catumaxomab), REVLIMID® (lenalidomide), RSR13 (efaproxiral), SOMATULINE® LA (lanreotide), SORIATANE® (acitretin), staurosporine (Streptomyces staurospores), talabostat (PT100), TARGRETIN® (bexarotene), TAXOPREXIN® (DHA-paclitaxel), TELCYTA® (canfosfamide, TLK286), temilifene, TEMODAR® (temozolomide), tesmilifene, thalidomide, THERATOPE® (STn-KLH), thymitaq (2-amino-3,4-dihydro-6-methyl-4-oxo-5-(4-pyridylthio)quinazoline dihydrochloride), TNFERADE™ (adenovector: DNA carrier containing the gene for tumor necrosis factor-α), TRACLEER® or ZAVESCA® (bosentan), tretinoin (Retin-A), tetrandrine, TRISENOX® (arsenic trioxide), VIRULIZIN®, ukrain (derivative of alkaloids from the greater celandine plant), vitaxin (anti-alphavbeta3 antibody), XCYTRIN® (motexafin gadolinium), XINLAY™ (atrasentan), XYOTAX™ (paclitaxel poliglumex), YONDELIS® (trabectedin), ZD-6126, ZINECARD® (dexrazoxane), ZOMETA® (zolendronic acid), zorubicin and the like.

In one embodiment, the formulation comprising the anti-EGFR-ADC is intravenously administered to a subject having glioblastoma in combination with radiation and/or TEMODAR® (temozolomide).

Further, in one embodiment, the composition of the invention can be provided as a pharmaceutical kit comprising (a) a container containing an anti-EGFR ADC in lyophilized form and (b) a second container containing a pharmaceutically acceptable diluent (e.g., sterile water) for injection. The pharmaceutically acceptable diluent can be used for reconstitution or dilution of the lyophilized ADC. Optionally associated with such container(s) can be a notice 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 invention is further described in the following examples, which are in not intended to limit the scope of the invention.

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EXAMPLES

Example 1: Conjugation of Auristatin vc-MMAE to Anti-EGFR Antibody 1

The following example describes the conjugation of an antibody to an auristatin to form an antibody drug conjugate (ADC), specifically the conjugation of MMAE to anti-EGFR Antibody 1. Generation of the Antibody 1 ADC with reduced drug loads of vc-MMAE molecules per antibody, involved a partial reduction of the mAb followed by reaction with Val-Cit-MMAE (vcMMAE) to complete the conjugation, as described in detail below.

Disulfide Reduction of Antibody

Reduction of Antibody 1 was achieved using TCEP (tricarboxyethyl phosphine). Recombinant monoclonal Antibody 1 was produced by a transfected Chinese hamster ovary (CHO) cell line and purified at Abbott Bioresearch Center (Worcester, MA). Following antibody purification, the antibody solution (148 mg/mL, 6 mL) was charged into a 50 mL polypropylene centrifuge tube. The antibody solution was then diluted to a total volume of 41 mL by adding PBSE Buffer (360 mL; 125 mM K_2HPO_4, 150 mM NaCl; 6.3 mM EDTA, pH 7.7). Protein content was 21.6 mg/ml as determined by A_{280}. 19 ml of antibody solution was charged into a reactor for a total of 410.6 mg. The antibody solution was warmed to 37 °C. Antibody 1 (20 mg/mL) was then partially reduced by the addition of TCEP (Sigma Aldrich Fine Chemical (St. Louis, MO)) to the antibody solution. Specifically, 9.67 mM TCEP solution (0.592 mL, 2.05 equiv) was added to the antibody solution (molar equivalents of TCEP :mAb was 2.05). Following the addition of TCEP, the antibody solution was incubated at 37°C for 1 hour. The reduction reaction was then chilled to 20°C. This process resulted in the reduction of the disulfide bonds of Antibody 1.

Conjugation of MMAE and Antibody 1

The following describes the process by which MMAE was conjugated to exemplary Antibody 1 following reduction of the antibody.

To conjugate the thiols of Antibody 1, Val-Cit-MMAE (vc-MMAE). Val-Cit (para-aminobenzylcarbamate-monomethylauristatin E; Sigma Aldrich Fine Chemical (St. Louis, MO)) was added to the antibody solution to a final vc-MMAE:reduced Cysteine (Cys) molar

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ratio of 1.15. The conjugation reaction was carried out in the presence of 10% v/v of DMSO (dimethylsulfoxide; Sigma Aldrich Fine Chemical (St. Louis, MO)), and allowed to proceed at 20°C for 45 minutes.

After the conjugation reaction, excess free N(acetyl)-Cysteine (Sigma Aldrich Fine Chemical (St. Louis, MO) (2.3 equivalents vs. vcMMAE charge) was added to quench unreacted vc-MMAE to produce N(acetyl)-Cys-vc-MMAE. The N(aceytl)-Cys quenching reaction was allowed to proceed at 20°C for approximately 30 minutes. The quenched reaction mixture (also referred to as the crude solution) was then purified, as described below in Example 2.

Alternatively, the following protocol was also used for a smaller scale reaction. conjugation was performed by charging 10mM vcMMAE DMSO solution (1.32 mL, 4.72 equiv.). Charge DMSO (0.86 mL). The reaction mixtures was then stirred at ambient temperature for 1 hour. Excess drug linker was quenched by the addition of 50 mM N-(acetyl) Cysteine (0.53 mL). The mixture was then stirred for about 15 minutes. The reaction mixture was then stored in the refrigerator.

Analytical Analysis of Reaction Mixture

Analytical analysis of the reaction mixture was performed. Analysis of the supernatant samples was accomplished by hydrophobic interaction chromatography–high-performance liquid chromatography (HIC-HPLC) using an TSKgel Butyl-NPR column (4.6 mm ID x 3.5 cm, 2.5 um;Tosoh Bioscience LLC, Japan)).

UV analysis of the protein content showed 386.4 mg of protein. HIC trace analysis showed that the average Drug to Antibody Ratio (DAR) for Antibody 1-vcMMAE was 3.85, as described below in Table 1. The average DAR was determined by summing up the 2, 4, 6 and 8 ADC product of multiplying PA% (PA% is the peak area percent as determined by the area measured under the peak at A_{280}) by requisite drug load and dividing by 100, e.g., [(6.3 PA% x 0) + (24.8 PA% x 2) + (34.8 x 4) + (20.9 x 6) + (8.8 x 8)] / 100 = 3.85.

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Table 1: HIC Trace Analysis of Reaction Mixture

HIC		
DAR	PA%	Drug Load Equivalent
0	6.34	0
2	24.84	49.680
4	34.84	139.360
6	20.92	125.520
8	8.79	70.320
Average DAR		3.8488

As described in Table 1, the conjugation reaction resulted in a mixture of species of ADCs having a range of drug loads, *i.e.,* DARs of 2 to 8. A small percentage of ADCs had no drug load as described in Table 1.

Example 2: Batch Purification of Antibody Drug Conjugate (ADC) Using a Hydrophobic Resin

The following example describes batch purification of an ADC (Antibody 1-vc-MMAE), where the resulting purified composition had an average DAR of 2.8. The following purification process selectively removed the higher loaded ADCs, *i.e.,* the six and eight drug-loaded species, resulting in a purified distribution comprising lower ordered drug load species, *i.e.*, DARs of 2-4. The purification process utilized small amounts of a hydrophobic resin that could be titrated in to the crude antibody solution (or mixture) in order to selectively remove ADCs of varying degree of conjugation.

The purification process provides a practical, scalable process to selectively modulate the distribution of both Auristatin E and Auristatin F conjugates resulting from partial inter-chain disulfide reduction and subsequent alkylation with vc-MMAE or mc-MMAF. The purification method described below has been demonstrated on both a milligram to multi-gram scale in either batch mode or in circulation mode affording the purified distribution in 86% yield. An overview of the antibody reduction, conjugation, and purification process is described in Figure 1.

Materials and Methods

The buffers described herein were prepared as follows:

Buffer A (50 mM K_2HPO_4 buffer pH 7 Buffer / 2M NaCl) was prepared by charging K_2HPO_4 (0.87 g) (K_2HPO_4; Fisher Scientific) and NaCl (11.7 g) (NaCl; EMD) diluting with WIFI to approximately 90 mL. The resulting solution was treated with 1.0 N HCl to a final pH of 7.0 and further diluted to a total volume of 100 mL.

Buffer A' (50 mM K_2HPO_4 / 4M NaCl) was prepared by charging NaCl (2.92 g) into a flask followed by charging Mobile Phase A to achieve a final volume of 25 mL.

Buffer B (50 mM K_2HPO_4 buffer pH 7 Buffer) was prepared by charging K_2HPO_4 (0.87 g) and NaCl (11.7 g) diluting with WIFI (water-intended for injection; Gibco) to approximately 90 mL. The resulting solution was treated with 1.0 N HCl (1.0 N HCl; JT Baker), to a final pH of 7.0 and further diluted to a total volume of 100 mL.

Pre-treated Butyl-HIC (Bu-HIC) resin was prepared by briefly mixing the bulk container of ToyoPearl Butyl - 600M Resin slurry (ToyoPearl Bu-HIC Resin (600M); Tosoh Bioscience), pouring out (1 gram) into a coarse polypropylene filter. The slurry was filtered and rinsed with Buffer A (3 x 2 mL). The wet cake was dried by passing filtered nitrogen through the wet cake for 10 minutes or until no more droplets were observed on the bottom of the coarse funnel. The dry weight basis was calculated by subtracting the amount of water present on the wet cake. The amount of moisture was measured by Karl Fisher analysis (typically contains 55% water).

Titration Screening Study to Determine Conditions for ADC DAR 2-4

A solid phase titration study was performed to determine the conditions for removing ADCs having a DAR of 6-8. Analysis of the supernatant samples was accomplished by hydrophobic interaction chromatography–high-performance liquid chromatography (HIC-HPLC) using an TSKgel Butyl-NPR column (4.6 mm ID x 3.5 cm, 2.5 um; Tosoh Bioscience LLC, Japan)). The method consisted of a linear gradient from 100% buffer A [25 mM sodium phosphate, 1.5 M $(NH_4)_2SO_4$, pH 7.0] to 100% buffer B [75% v/v 25 mmol/L sodium phosphate (pH 7.0), 25% v/v isopropanol] in 12 minutes. The flow rate was set at 0.8 mL/min, inject 30 uL, the temperature was set at 30°C, and detection was followed at 280 nm.

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Sample Preparation for HIC-HPLC Analysis

The sample was prepared by filtering off the reaction mixture slurry through a 5 um syringe filter. The filtrate was diluted 5-fold with Buffer A (30 uL/injection).

Eight conditions (Assays-Bu-0, Bu-1, Bu-2, Bu-4, Bu-8, Bu-16, Bu-32, and scale up) were tested with varying amounts of resin in each. Assay-Bu-0 was performed by charging 100 uL of crude Antibody 1-vcMMAE reaction solution (Example 1) (18 mg/mL) into a vial. Buffer A' (100 uL) was added, followed by the addition of 100 uL of Buffer B. The solution was then shaken on the lowest setting (orbital mixer). A sample of the supernatant was taken at 20 minutes. Supernatant was sampled and measured according to HIC-HPLC. Specifically, sample preparation was conducted by removing 30 uL of the supernatant, diluting it with 120 uL of Buffer A, and measuring the contents by HIC-HPLC. See Table 5 for a summary of distribution.

Assays-Bu-1 to Bu-32 were all variants of Assay-Bu-0, which did not contain any hydrophobic interaction resin and was the control. Assay-Bu-1 was the same as Assay-Bu-0 except 0.8 mg of preconditioned n-Bu HIC 600M resin was added to the solution prior to adding Buffer B. Assay-Bu-2 was the same as Assay-Bu-0 except 1.6 mg of preconditioned n-Bu HIC 600M resin was added prior to adding Buffer B. Assay-Bu-4 was the same as Assay-Bu-0 except 3.2 mg of preconditioned n-Bu HIC 600M resin was added prior to adding Buffer B. Assay-Bu-8 was the same as Assay-Bu-0 except 6.4 mg of preconditioned n-Bu HIC 600M resin was added prior to adding Buffer B. Assay-Bu-16 was the same as Assay-Bu-0 except 12.8 mg of preconditioned n-Bu HIC 600M resin was added prior to adding Buffer B. Assay-Bu-32 was the same as Assay-Bu-0 except 25.6 mg of preconditioned n-Bu HIC 600M resin was added prior to adding Buffer B. For each of these experiments, the final NaCl concentration was 1.3 M NaCl. The results from the eight conditions are summarized in Tables 2-5 below.

Table 2 provides a summary of the distribution of various ADC species (e.g., antibody alone / unconjugated (% mAb), an ADC having a DAR of 2 (%2 Load), an ADC having a DAR of 4 (% 4 Load), etc.). The load to protein ratio described in Table 2 represents the dry weight of the resin vs. the calculated antibody protein weight. ADC weight is calculated by total protein content as measured by UV absorption at 280 nm multiplied by peak area% of

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the drug loaded species. As described in Table 2, the resin load:protein ratio impacted the % ADC having certain DARs. For example, a resin load vs. total protein of 1.8 (or 5.9 weights of resin versus the 6-8 load; see row "Assay-Bu-4" of Table 2) resulted in 94% purity of the 2 or 4 Drug-loaded Species (47% each) and no detectable level of ADCs having a DAR of 6 or 8, as determined by HIC-HPLC. Table 3 describes the amount of Bu-HIC resin that was added for each experiment described in Table 2, while Table 4 describes the calculation of net weight of drug loaded species present in the screen.

Table 2. Summary of Distribution as Measured by HIC-HPLC

Experiment	Resin Load vs Total Protein	Resin load vs. 6-8	% mAb	%2 Load	% 4 Load	% 6 Load	% 8 Load	Average DAR
Assay-Bu-0*	**0**	0	3.4	30.1	36.6	21.8	8.1	4.02
Assay-Bu-1*	**0.44**	1.5	4.4	31	40.3	22	2.2	3.73
Assay-Bu-2*	**0.9**	3.0	4.4	37	47.3	4.3	0	2.89
Assay-Bu-4*	**1.8**	5.9	5.9	47	47	0	0	2.82
Assay-Bu-8*	**3.5**	11.7	10.4	74.2	15.5	0	0	2.10
Assay-Bu-16	**7.1**	23.7	59.3	40.7	0	0	0	0.81
Assay-Bu-32	**14.2**	47.5	100	0	0	0	0	0
Assay (Scale-up)	**2**	6.7	8.7	40	47	1.5	0	2.82

* As measured at > 24 hours of residence time with addition of 1 volume% of IPA

Table 3: Charge Amounts of Bu-HIC Resin

Experiment	Weight of Total Protein in mgs in vial**	Wet Weight* Bu-Resin Charge (mg)	Dry Weight Bu-resin Charge (mg)	Dry Weight Resin / Weight of Total Protein	Dry weight vs. 6-8
Assay-Bu-0	1.8	0	0	0	0
Assay-Bu-1	1.8	1.8	0.8	0.44	1.5
Assay-Bu-2	1.8	3.6	1.6	0.9	3.0
Assay-Bu-4	1.8	7.2	3.2	1.8	6.0
Assay-Bu-8	1.8	14.4	6.4	3.5	11.7
Assay-Bu-16	1.8	28.8	12.8	7.1	23.7
Assay-Bu-32	1.8	57.6	25.6	14.2	47.5

*(water content of resin = 55%); ** Based on 280 nm protein concentration and volume delivered per vial

Table 4: Calculation of Net Weight of Drug Loaded Species Present in Screen

Drug Load Species	PA%* of Drug Load Species	Calculated Net Weight of Drug Load Species** (mg) in vial
0-Load (mAb)	2.8	0.05
2-Load	29.8	0.54
4-Load	37.3	0.67
6-Load	22.3	0.40
8-Load	7.8	0.14
6/8 Load	30.1	0.54
4/6/8 Load	67.4	1.21
2/4/6/8 Load	97.2	1.75
0/2/4/6/8	100	1.8

* Odd Species were not included; ** 1.8 mg total protein / vial

The above analytical HIC results demonstrated sufficient selectivity to reduce the presence of certain drug loaded species (e.g., ADCs having a DAR of 6-8). The data from Tables 2 and 5 suggest that, in the presence of an end ionic strength of ~ 1.3 M NaCl concentration, or an equivalent ionic strength thereof, reduction of the 8-loaded species can largely be achieved by utilizing a 0.5 (specifically 0.44% wt) wt resin charge; the reduction of the 6/8 loaded species can be achieved through the addition of about two weights of hydrophobic resin to crude conjugation reaction mixture. The 4-8 drug loaded species can largely be removed by using ~ 3.5 weights of resin (versus total protein (see "Assay-Bu-8" row of Table 5) and the 2-8 loaded species can be removed by utilizing ~ 7 weights of resin versus total protein (see "Assay-Bu-16" row of Table 5) . These data also suggest that the 4 weight load or 2 weight loading (versus total protein) on a dry weight basis was selective at removing the high drug loads, with minimal attrition of the 2 or 4 drug loaded species.

The weight ratio loading of resin to species to be reduced was calculated and summarized in Table 5.

Summaries of the analytical HIC study results are provided in Table 5.

Table 5: Summary of Drug Loaded Species* vs. Resin Charge

Experiment	Net weight of resin in mg (dry Weight)	Weights of Resin (dry Weight) vs. 8 Load (0.14 mg/vial)	Weights of Resin 6/8 Load (0.54 mg/vial)	Weights of Resin 4/6/8 Load (1.21 mg/vial)	Weights of Resin 2/4/6/8 Load (1.75 mg/vial)	Weights of resin / Total Protein (1.8 mg/vial)
Assay-Bu-0	0	0	0	0	0	0
Assay-Bu-1	0.8	5.7	1.5	0.7	0.5	**0.44**
Assay-Bu-2	1.6	11.5	3.0	1.3	0.9	0.9
Assay-Bu-4	3.2	22.9	**5.9**	2.6	1.8	1.8
Assay-Bu-8	6.4	45.7	11.7	5.3	3.7	3.5
Assay-Bu-16	12.8	91.4	23.7	10.6	7.3	7.1
Assay-Bu-32	25.6	183	47.5	21.2	14.7	14.2

** See Table 3 for calculation of drug load species*

** Underlined values denote approximate amount of resin load where the amount of 6/8 species was less than the 2% (as shown in Table 2)

Summary

In sum, the reaction mixture obtained from Example 1 was diluted with Buffer A' (4N NaCl, 0.05 M pH 7 K_2HPO_4 phosphate buffer, 1 mL/mL conjugation reaction mixture). The diluted reaction mixture was treated with the calculated amount of pre-treated Bu-HIC resin, filtered through a coarse polypropylene filter, and further diluted with Buffer B (0.05 M pH 7 K_2HPO_4 phosphate buffer, 1 mL/ mL conjugation reaction mixture). The calculated amount of resin, as shown in Table 5, depends on the drug load species that is to be removed from the crude mixture. For example, a resin weight of approximately 5 to 10 times that of the 6 and 8 drug load species was proven to be effective for removing these species from the crude mixture. The resin / diluted reaction mixture was stirred for the appropriate time, and monitored by analytical hydrophobic interaction chromatography for reduction of the specified drug conjugate products.

Example 3: Scale Up of Batch Hydrophobic Interaction ADC Purification

The optimal conditions for removing high DAR ADCs that were identified from the titration screen were scaled up for larger scale purification.

In order to first reduce the antibody, a solution containing Antibody 1 (151 mg/mL, 50 mL, 7.52g) was added to a 500 mL flask. The solution was diluted to a total volume of 395 mL by the addition of a solution prepared by mixing a pH 6, 15 mM Histidine buffer (30 mL) and PBSE Buffer (360 mL; 125 mM K_2HPO_4, 150 mM NaCl; 6.3 mM EDTA, pH 7.7). The resulting antibody solution was warmed to 37° C. 10.98 mM TCEP solution (12.1 mL, 2.05 equiv) was then added to the solution, which was stirred for 30 minutes. The antibody solution was then cooled to ambient temperature over 20 minutes.

Once reduced, Antibody 1 was conjugated to vcMMAE by adding 10mM vcMMAE DMSO solution (28.8 mL, 4.72 equiv.) to the antibody solution. DMSO (21.2 mL) was added next, whereupon the solution was stirred at ambient temperature for 45 minutes. Excess drug linker was quenched by the addition of 50 mM N-Acetyl cysteine (9.7 mL). The solution was stirred for about 15 minutes. UV protein concentration was determined to be 7.4 g of protein

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following the conjugation of Antibody 1 to vcMMAE, and HIC analysis showed a DAR of 4.1 (about a 25 PA% (or 1.85 g) combined 6-8 drug load species).

The crude reaction mixture was then diluted with an equal volume of 4N NaCl / 0.05 M pH 7 K_2HPO_4 buffer. 30.7 g pre-washed Bu HIC wet resin (KF = 56 wt% water; net 17.2 g resin on dry weight basis; 2.3 weights resin vs total protein; 9.3 weights resin vs 6-8 loaded species) was then added, followed by the addition of 50 mM KH_2PO_4 pH7 Buffer (460 mL). The antibody resin solution was gently stirred for 3 hours at room temperature. Alternatively, the solution was stored for 12 hours in the refrigerator, and subsequently stirred for an additional 2.5 hours.

Resin was then filtered off through a coarse polypropylene filter. Clear filtrate was poured into a new container. Weight of 1469 g (density = 1.06 g/mL) was determined. Analytical analysis of purified ADC solution showed that the solution had a UV protein content of 3.7 mg/ml or 5.07 g of total protein (a 67% overall yield). The HIC trace analysis showed a DAR of 2.8.

A graph showing an overlay of HIC-HPLC of the antibody solutions before and after purification is provided in Figure 2. The two late eluting peaks in Figure 2 represent ADCs having a DAR of 6-8 (retention time: 8.6 minutes and 9.6 minutes respectively). These peaks are missing following purification, demonstrating that the 0, 2, and 4 DAR ADC species are not affected and that this purification process is selective in that it removes only the high (e.g., 6 – 8) DAR ADC species.

UF/DF (Concentration and Final Buffer Exchange)

Following purification, the purified ADC solution was subjected to ultrafiltration / diafiltration (UF/DF) and final buffer exchange. The filtrate was added to the UF/DF reservoir, concentrating the solution to ~ 50 mg/mL and removing 1400 g on a Pall Centramate Omega 30K LV1 part OS030C12P1 serial number 31061058R at a transmembrane pressure of ~ 25 psi and a peristaltic pump speed of 80 – 100 mL/minute (approximately 1 hour to concentrate). After concentrating to ~ 50 mg/mL, 10 DV of a 15 mM pH 6.0 Histidine buffer was run. The UF/DF system was drained and subsequently flushed with 15 mM pH 6.0 Histidine buffer (2 x 20 mL). Concentration was measured at (127 g solution) 40.1 mg/mL. Diluted with 15 mM Histidine pH6.0 buffer to a concentration

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of 35.1 mg/mL (141 g BDS). The BDS was filtered through 0.45 micron syringe filter, followed by a 0.2 micron sterile filtration. Filtrate was charged into 12 vials at 100 mg each and Falcon tubes (3 x 35 mL).

The above UF/DF process may be used prior to or following the batch purification process.

In a separate experiment, 2 grams of pre-treated Toyopearl Butyl-600M HIC resin (Tosoh Bioscience, Japan)/ 1 gram of mAb and further dilution with 0.05 M pH 7 K_2HPO_4 phosphate buffer and stirred at ambient for 6 hours. The slurry was filtered through a coarse polypropylene filter and washed with Buffer A (2 wet cake bed volumes). The resulting filtrate and rinses were combined and buffer-exchanged into 0.015 M pH 6 histidine buffer by diafiltration to afford the purified Antibody 1-Val-Cit-MMAE referred to as Antibody 1-vcMMAEp. In this example, the overall yield was 67% of Antibody 1-vcMMAEp. Based on the amount of 0-4 drug loaded species in the crude reaction mixture, the purification yield was 87%.

Example 4: Preparation of Anti-EGFR Antibody 1 / mc-MMAF ADC

The following example describes the preparation of Anti-EGFR Antibody 1 mc-MMAF ADC.

Reduction of Anti-EGFR Antibody 1

Following antibody purification, the antibody solution (151 mg/mL, 86 mL) was charged into a 1 L flask. The antibody solution was then diluted to a total volume of 729 mL by adding PBSE Buffer (600 mL; 125 mM K_2HPO_4, 150 mM NaCl; 6.3 mM EDTA, pH 7.7) and 15 mM Histidine buffer (43 mL, pH 6). Protein content was 20.0 mg/ml as determined by UV spectroscopy (A_{280}). The solution containing Antibody 1 was heated to 37°C. A 9.67 mM TCEP solution (0.592 mL, 2.05 equiv) was then added to the solution of Antibody 1 under stirring for 30 minutes. The reaction was subsequently cooled to ambient temperature over 20 minutes.

Conjugation of mcMMAF to Anti-EGFR Antibody 1

Anti-EGFR Antibody 1 was subsequently conjugated to maleimidocaproyl-MMAF (Antibody 1-mcMMAF). Charge 10 mM mcMMAF/DMSO (38 mL, 4.72 equivalents). Charge DMSO (18.6 mL). Stir for 1 hour at ambient temperature. Excess mc-MMAF was quenched by the addition of 100 mM N-Acetyl cysteine (7.6 mL) and stirring for 15 minutes. The quenched reaction was placed in the refrigerator.

Analysis of the Crude Antibody 1-mcMMAF Reaction Mixture

The reaction mixture was analyzed to determine the protein concentration. UV spectroscopy (A_{280}) showed a protein concentration of 17.7 mg/mL. Analysis of the resulting HIC trace revealed a DAR of 3.93 (Table 6). The average DAR was determined by summation of the 2, 4, 6 and 8 ADC product of multiplying PA% by requisite drug load) and dividing by 100, e.g., [(5.72 PA% x 0) + (27.27 PA% x 2) + (41.08 x 4) + (16.79 x 6) + (9.13 x 8)] / 100 = 3.93.

Table 6: HIC Trace Analysis of Antibody 1-mc-MMAF Reaction Mixture

DAR	PA%	Drug Load
0	5.72	0
2	27.27	49.680
4	41.08	139.360
6	16.79	125.520
8	9.13	70.320
Average DAR*		3.93

* Summation of the Drug Load Equivalents / 100

As described in Table 6, the conjugation reaction resulted in a mixture of species of ADCs having a range of drug loads, *i.e.,* DARs of 2 to 8. A small percentage of ADCs had no drug load as described in Table 6.

UF/DF (Concentration and Final Buffer Exchange)

The purified ADC solution was subjected to ultrafiltration /diafiltration (UF/DF) and final buffer exchange. Tangential flow filtration was performed on a Millipore Biomax Pellicon 3 88 cm^2 membrane. The sample was concentrated to 100 mg/mL at 20 psi (TMP)

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and 40 mL/min crossflow. The protein was subsequently diluted to a concentration of 60 mg/mL with 15 mM Histidine buffer (pH 6) by performing 10 DVs at approximately 20 psi (TMP) at a rate of 40 mL/min. The resulting solution was filtered through a 0.45 μm Millipak 20 filter (Millipore) . The protein concentration was determined to be 59.7 mg/mL via UV spectroscopy (A_{280}). A 58.6 mL sample of the UF/DF purified bulk mc-MMAF Antibody 1 solution was subsequently diluted with 15 mM Histidine buffer (pH 6.0) to a final volume of 100 mL. The concentration as determined by UV spectroscopy was 35.7 mg/mL. The protein solution was then filtered through a 0.2 μm Millipak 20 filter (Millipore) into a sterile 125 ml PETG bottle. The purified mc-MMAF Antibody 1 solution was frozen and stored in a -80°C cryofreezer.

Example 5: Batch Purification of mc-MMAF ADC Using Hydrophobic Resin

The purified Antibody 1-mc-MMAF from Example 4 was subjected to a resin treatment purification screen. The screen was performed by varying the total resin charge (0.5, 1, 2, and 3 wts; the purified Antibody 1-mc-MMAF varied from 9.5 mg/mL to 34 mg/mL), the NaCl concentration (0 N, 0.65 N, 1.3 N), and the residence time (0.5 hours, 4 hours, and 20 hours). Table 7 provides a summary of the distribution of the various ADC species as a function of the resin charge, NaCl concentration, and residence time. The DAR values were determined by analysis of the HIC trace as described above. The calculated yield was determined by UV spectroscopy and are summarized in Table 8.

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Table 7: Summary of Distribution as Measured by HIC-HPLC

Description	Resin Load vs. Total Protein	Resin Loading vs. 6-8 drug Load	Residence Time (Hours)	Salt Concentration (Molarity of NaCl)	0 Load	2 Load	4 Load	6 Load	8 Load	DAR	Calculated Yield (280 nm)
T0 0N NaCl	0	0	0	0	5	28.1	43.2	17	6.7	3.85	N/A
T0 0.6N NaCl	0	0	0	0.65	5.1	29.1	42.6	16.6	6.6	3.81	N/A
T0 1.3N NaCl	0	0	0	1.3	5.1	29.7	42.3	16.5	6.5	3.8	N/A
M30 0N NaCl 0.5 wt Resin	0.5	2.1	0.5	0	5.2	30	43.5	16.2	5	3.71	97%
M30 0N NaCl 1 wt Resin	1	4.2	0.5	0	5.5	31.3	44.4	15.4	3.4	3.6	94%
M30 0N NaCl 2 wt Resin	2	8.4	0.5	0	6.1	35.2	47.8	10.5	0.4	3.28	85%
M30 0N NaCl 3 wt Resin	3	12.6	0.5	0	6.7	38.2	48.9	6.2	0	3.09	78%
M30 0.6N NaCl 0.5 wt Resin	0.5	2.1	0.5	0.65	5.4	31.2	44.7	15.8	2.9	3.59	90%
M30 0.6N NaCl 1 wt Resin	1	4.2	0.5	0.65	5.9	34.5	47.8	11.2	0.6	3.32	86%
M30 0.6N NaCl 2 wt Resin	2	8.4	0.5	0.65	6.7	38.7	50.1	4.5	0	3.05	74%
M30 0.6N NaCl 3 wt Resin	**3**	**12.6**	**0.5**	**0.65**	**8**	**44.4**	**46.5**	**1.1**	**0**	**2.81**	**63%**
M30 1.3N NaCl 0.5 wt Resin	0.5	2.1	0.5	1.3	5.5	33.2	46	13.8	1.4	3.44	93%
M30 1.3N NaCl	1	4.2	0.5	1.3	6.2	35.8	49	9	0	3.22	81%

1 wt Resin											
M30 1.3N NaCl 2 wt Resin	**2**	**8.4**	**0.5**	**1.3**	**7.7**	**42**	**48.1**	**2.2**	**0**	**2.9**	**68%**
M30 1.3N NaCl 3 wt Resin	**3**	**12.6**	**0.5**	**1.3**	**9.4**	**52.4**	**38.2**	**0**	**0**	**2.58**	**55%**
H4 0N NaCl 0.5 wt Resin	0.5	2.1	4	0	5.3	30	44.2	16.2	4.3	3.68	1%
H4 0N NaCl 1 wt Resin	1	4.2	4	0	5.6	31.3	46.7	14.6	1.8	3.51	90%
H4 0N NaCl 2 wt Resin	2	8.4	4	0	6.4	36.6	48.9	8.1	0	3.17	84%
H4 0N NaCl 3 wt Resin	3	12.6	4	0	7.2	40.3	48.4	4	0	2.98	72%
H4 0.6N NaCl 0.5 wt Resin	0.5	2.1	4	0.65	5.6	32	45.7	14.8	1.9	3.51	92%
H4 0.6N NaCl 1 wt Resin	1	4.2	4	0.65	6.1	34.6	48.5	10.8	0	3.28	86%
H4 0.6N NaCl 2 wt Resin	**2**	**8.4**	**4**	**0.65**	**7.3**	**40.8**	**49**	**2.8**	**0**	**2.94**	**70%**
H4 0.6N NaCl 3 wt Resin	**3**	**12.6**	**4**	**0.65**	**8.8**	**49.4**	**41.8**	**0**	**0**	**2.66**	**59%**
H4 1.3N NaCl 0.5 wt Resin	0.5	2.1	4	1.3	5.6	33.3	46.7	13.6	0.8	3.41	88%
H4 1.3N NaCl 1 wt Resin	1	4.2	4	1.3	6.2	36	49.3	8.4	0	3.2	80%
H4 1.3N NaCl 2 wt Resin	**2**	**8.4**	**4**	**1.3**	**7.8**	**44.7**	**47.1**	**0.5**	**0**	**2.81**	**64%**
H4 1.3N NaCl 3 wt Resin	**3**	**12.6**	**4**	**1.3**	**10.6**	**56.1**	**33.4**	**0**	**0**	**2.46**	**48%**
H20 0N NaCl	0.5	2.1	20	0	5.1	29.4	44.6	16.4	4.4	3.71	100%

0.5 wt Resin											
H20 0N NaCl 1 wt Resin	1	4.2	20	0	5.4	31.4	47.1	14.3	1.8	3.51	95%
H20 0N NaCl 2 wt Resin	2	8.4	20	0	6.1	34.7	50	9.1	0	3.24	85%
H20 0N NaCl 3 wt Resin	3	12.6	20	0	7.3	40.5	48.4	3.8	0	2.97	70%
H20 0.6N NaCl 0.5 wt Resin	0.5	2.1	20	0.65	5.5	28.9	47.2	16.3	2.1	3.61	91%
H20 0.6N NaCl 1 wt Resin	1	4.2	20	0.65	5.9	33.9	49.7	10.5	0	3.3	83%
H20 0.6N NaCl 2 wt Resin	**2**	**8.4**	**20**	**0.65**	**7.2**	**39.7**	**50.6**	**2.5**	**0**	**2.97**	**71%**
H20 0.6N NaCl 3 wt Resin	**3**	**12.6**	**20**	**0.65**	**9.2**	**49**	**41.8**	**0**	**0**	**2.65**	**58%**
H20 1.3N NaCl 0.5 wt Resin	0.5	2.1	20	1.3	7.1	45.9	37.8	9.1	0	2.98	90%
H20 1.3N NaCl 1 wt Resin	1	4.2	20	1.3	6.2	35	50	8.8	0	3.23	77%
H20 1.3N NaCl 2 wt Resin	**2**	**8.4**	**20**	**1.3**	**7.8**	**43.8**	**48.4**	**0**	**0**	**2.81**	**67%**
H20 1.3N NaCl 3 wt Resin	**3**	**12.6**	**20**	**1.3**	**10.8**	**56.9**	**32.3**	**0**	**0**	**2.43**	**47%**

Bold font in Table 7 indicates conditions that removed 6-loaded species < 3 PA% (peak area%); the 8-loaded species were undetectable under these experimental conditions. "T0" refers to a residence time of 0 minutes (i.e. control experiment with no resin); "M30" refers to a residence time of 0.5 hours; "H4" refers to a residence time of 4 hours; "H20" refers to a residence time of 20 hours.

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Table 8: UV Antibody 1-mc-MMAF Concentration Results as a Function of the Resin Treatment Purification Screen

0.5 Hours Residence Time	0.5 Hours Residence Time	**Protein Recovery of 0 N NaCl**	**Protein Recovery of 0.6 N NaCl**	**Protein Recovery of 1.3 N NaCl**
Resin Loading (Dry Weight Basis) vs. Protein Load	**Resin Loading (Dry Weight Basis) vs 6-8 Load**			
0	**[0]**	**100%**	**99%**	**98%**
0.5	[2.1]	**97%**	**90%**	**93%**
1	[4.2]	**94%**	**86%**	**81%**
2	[8.4]	**85%**	**74%**	**68%**
3	[12.6]	**78%**	**63%**	**55%**
4 Hours Residence Time	4 Hours Residence Time			
Resin Loading (Dry Weight Basis) vs. Protein Load	**Resin Loading (Dry Weight Basis) vs 6-8 Load**			
0	0	**100%**	**99%**	**98%**
0.5	2.1	**101%**	**92%**	**88%**
1	4.2	**90%**	**86%**	**80%**
2	8.4	**84%**	**70%**	**64%**
3	12.6	**72%**	**59%**	**48%**
20 Hours Hour Residence Time	20 Hours Hour Residence Time			
Resin Loading (Dry Weight Basis) vs. Protein Load	**Resin Loading (Dry Weight Basis) vs 6-8 Load**			
0	0	**100%**	**99%**	**98%**
0.5	2.1	**100%**	**91%**	**90%**
1	4.2	**95%**	**83%**	**77%**
2	8.4	**85%**	**71%**	**67%**
3	12.6	**70%**	**58%**	**47%**

In sum, the resin titration screens describes in Tables 7 and 8 were performed to determine the impact of the resin load, NaCl concentration, and residence time on the purification process (DAR, protein concentration) obtained from the UF/DF purified Antibody 1-mc-MMAF ADC from Example 4. . The calculated amount of resin as shown in Table 7, depends on the drug load species to be removed from the crude distribution. A series of reaction conditions using Antibody 1-mcMMAF from Example 4 were tested as described below (referred to in Tables 7 and 8). Buffer A contains the following: 4.35g K_2HPO_4; 58.5g NaCl; 495 mL water (WFI); pH adjusted to 7.0 with 5 mL 1N HCl.

Reaction 1: 0 N NaCl; No Resin:

1) Charge Antibody 1-mcMMAF (1.00 mL, 35 mg) into a 4 mL vial

2) Charge 0 mg Bu-HIC Resin

3) Shake

4) Pull supernatant sample at 0.5, 4, and 20 hours (remove 50 μL through syringe filter, dilute 20 μL of this supernatant sample with 2N NaCl / 0.05 M K_2HPO_4 pH 7 buffer (Buffer A) to afford a 1/50x).

5) Assay for UV protein concentration and perform HIC analysis.

Reaction 2: 0 N NaCl; 0.5 wt Resin:

1) Charge Antibody 1-mcMMAF (1.00 mL, 35 mg) into a 4 mL vial

2) Charge 17.5 mg Bu-HIC Resin

3) Shake

4) Pull supernatant sample at 0.5, 4, and 20 hours (remove 50 μL through syringe filter, dilute 20 μL of this supernatant sample with 2N NaCl / 0.05 M K_2HPO_4 pH 7 buffer (Buffer A) to afford a 1/50x).

5) Assay for UV protein concentration and perform HIC analysis.

Reaction 3: 0 N NaCl; 1 wt Resin:

1) Charge Antibody 1-mcMMAF (1.00 mL, 35 mg) into a 4 mL vial

2) Charge 350 mg Bu-HIC Resin

3) Shake

4) Pull supernatant sample at 0.5, 4, and 20 hours (remove 50 μL through syringe filter, dilute 20 μL of this supernatant sample with 2N NaCl / 0.05 M K_2HPO_4 pH 7 buffer (Buffer A) to afford a 1/50x).

5) Assay for UV protein concentration and perform HIC analysis.

Reaction 4: 0 N NaCl; 2 wt Resin:

1) Charge Antibody 1-mcMMAF (1.00 mL, 35 mg) into a 4 mL vial

2) Charge 70 mg Bu-HIC Resin

3) Shake

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4) Pull supernatant sample at 0.5, 4, and 20 hours (remove 50 µL through syringe filter, dilute 20 µL of this supernatant sample with 2N NaCl / 0.05 M K_2HPO_4 pH 7 buffer (Buffer A) to afford a 1/50x).

5) Assay for UV protein concentration and perform HIC analysis.

Reaction 5: 0 N NaCl; 3 wt Resin:

1) Charge Antibody 1-mcMMAF (1.00 mL, 35 mg) into a 4 mL vial

2) Charge 105 mg Bu-HIC Resin

3) Shake

4) Pull supernatant sample at 0.5, 4, and 20 hours (remove 50 µL through syringe filter, dilute 20 µL of this supernatant sample with 2N NaCl / 0.05 M K_2HPO_4 pH 7 buffer (Buffer A) to afford a 1/50x).

5) Assay for UV protein concentration and perform HIC analysis.

Reaction 6: 0.65 N NaCl; No Resin:

1) Charge Antibody 1-mcMMAF (1.00 mL, 35 mg) into a 4 mL vial

2) Charge 0.195 mL of a 4 N NaCl / 0.05 M K_2HPO_4 pH 7 buffer *(Buffer A').*

3) Charge 0 mg Bu-HIC Resin

4) Shake

5) Pull supernatant sample at 0.5, 4, and 20 hours (remove 50 µL through syringe filter, dilute 20 µL of this supernatant sample with 2N NaCl / 0.05 M K_2HPO_4 pH 7 buffer (Buffer A) to afford a 1/50X).

6) Assay for UV protein concentration and perform HIC analysis.

Reaction 7: 0.65 N NaCl; 0.5 wt Resin:

1) Charge Antibody 1-mcMMAF (1.00 mL, 35 mg) into a 4 mL vial

2) Charge 0.195 mL of a 4 N NaCl / 0.05 M K_2HPO_4 pH 7 buffer *(Buffer A').*

3) Charge 17.5 mg Bu-HIC Resin

4) Shake

5) Pull supernatant sample at 0.5, 4, and 20 hours (remove 50 µL through syringe filter, dilute 20 µL of this supernatant sample with 2N NaCl / 0.05 M K_2HPO_4 pH 7 buffer (Buffer A) to afford a 1/50X).

6) Assay for UV protein concentration and perform HIC analysis.

Reaction 8: 0.65 N NaCl; 1 wt Resin:

1) Charge Antibody 1-mcMMAF (1.00 mL, 35 mg) into a 4 mL vial

2) Charge 0.195 mL of a 4 N NaCl / 0.05 M K_2HPO_4 pH 7 buffer *(Buffer A').*

3) Charge 35 mg Bu-HIC Resin

4) Shake

5) Pull supernatant sample at 0.5, 4, and 20 hours (remove 50 µL through syringe filter, dilute 20 µL of this supernatant sample with 2N NaCl / 0.05 M K_2HPO_4 pH 7 buffer (Buffer A) to afford a 1/50X).

6) Assay for UV protein concentration and perform HIC analysis.

Reaction 9: 0.65 N NaCl; 2 wt Resin:

1) Charge Antibody 1-mcMMAF (1.00 mL, 35 mg) into a 4 mL vial

2) Charge 0.195 mL of a 4 N NaCl / 0.05 M K_2HPO_4 pH 7 buffer *(Buffer A').*

3) Charge 70 mg Bu-HIC Resin

4) Shake

5) Pull supernatant sample at 0.5, 4, and 20 hours (remove 50 µL through syringe filter, dilute 20 µL of this supernatant sample with 2N NaCl / 0.05 M K_2HPO_4 pH 7 buffer (Buffer A) to afford a 1/50X).

6) Assay for UV protein concentration and perform HIC analysis.

Reaction 10: 0.65 N NaCl; 3 wt Resin:

1) Charge Antibody 1-mcMMAF (1.00 mL, 35 mg) into a 4 mL vial

2) Charge 0.195 mL of a 4 N NaCl / 0.05 M K_2HPO_4 pH 7 buffer *(Buffer A').*

3) Charge 105 mg Bu-HIC Resin

4) Shake

5) Pull supernatant sample at 0.5, 4, and 20 hours (remove 50 µL through syringe filter, dilute 20 µL of this supernatant sample with 2N NaCl / 0.05 M K_2HPO_4 pH 7 buffer (Buffer A) to afford a 1/50X).

6) Assay for UV protein concentration and perform HIC analysis.

Reaction 11: 1.3 N NaCl; No Resin:

1) Charge Antibody 1-mcMMAF (1.00 mL, 35 mg) into a 4 mL vial

2) Charge 0.48 mL of a 4 N NaCl/0.05 M K_2HPO_4 pH 7 Buffer *(Buffer A').*

3) Charge 0 mg Bu-HIC Resin

4) Shake

5) Pull supernatant sample at 0.5, 4, and 20 hours (remove 50 µL through syringe filter, dilute 20 µL of this supernatant sample with 2N NaCl / 0.05 M K_2HPO_4 pH 7 buffer (Buffer A) to afford a 1/50X).

6) Assay for UV protein concentration and perform HIC analysis.

Reaction 12: 1.3N NaCl; 0.5 wt Resin:

1) Charge Antibody 1-mcMMAF (1.00 mL, 35 mg) into a 4 mL vial

2) Charge 0.48 mL of a 4 N NaCl/0.05 M K_2HPO_4 pH 7 Buffer *(Buffer A').*

3) Charge 17.5 mg Bu-HIC Resin

4) Shake

5) Pull supernatant sample at 0.5, 4, and 20 hours (remove 50 µL through syringe filter, dilute 20 µL of this supernatant sample with 2N NaCl / 0.05 M K_2HPO_4 pH 7 buffer (Buffer A) to afford a 1/50X).

6) Assay for UV protein concentration and perform HIC analysis.

Reaction 13: 1.3N NaCl; 1 wt Resin:

1) Charge Antibody 1-mcMMAF (1.00 mL, 35 mg) into a 4 mL vial

2) Charge 0.48 mL of a 4 N NaCl/0.05 M K_2HPO_4 pH 7 Buffer *(Buffer A').*

3) Charge 35 mg Bu-HIC Resin

4) Shake

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5) Pull supernatant sample at 0.5, 4, and 20 hours (remove 50 µL through syringe filter, dilute 20 µL of this supernatant sample with 2N NaCl / 0.05 M K_2HPO_4 pH 7 buffer (Buffer A) to afford a 1/50X).

6) Assay for UV protein concentration and perform HIC analysis.

Reaction 14: 1.3N NaCl; 2 wt Resin:

1) Charge Antibody 1-mcMMAF (1.00 mL, 35 mg) into a 4 mL vial

2) Charge 0.48 mL of a 4 N NaCl/0.05 M K_2HPO_4 pH 7 Buffer *(Buffer A').*

3) Charge 70 mg Bu-HIC Resin

4) Shake

5) Pull supernatant sample at 0.5, 4, and 20 hours (remove 50 µL through syringe filter, dilute 20 µL of this supernatant sample with 2N NaCl / 0.05 M K_2HPO_4 pH 7 buffer (Buffer A) to afford a 1/50X).

6) Assay for UV protein concentration and perform HIC analysis.

Reaction 15: 1.3N NaCl; 3 wt Resin:

1) Charge Antibody 1-mcMMAF (1.00 mL, 35 mg) into a 4 mL vial

2) Charge 0.48 mL of a 4 N NaCl/0.05 M K_2HPO_4 pH 7 Buffer *(Buffer A').*

3) Charge 105 mg Bu-HIC Resin

4) Shake

5) Pull supernatant sample at 0.5, 4, and 20 hours (remove 50 µL through syringe filter, dilute 20 µL of this supernatant sample with 2N NaCl / 0.05 M K_2HPO_4 pH 7 buffer (Buffer A) to afford a 1/50X).

6) Assay for UV protein concentration and perform HIC analysis.

Resin Preparation and Calculations:

Karl Fischer (KF) titration of resins:

Butyl = 72 wt% water (28% potency). Note all charges of resin are based on a dry weight basis.

A sample calculation of dry weight resin charge: 35 mg resin charge/0.28 dry weight potency= 125 mg of wet resin charge.

Generally, the potency of resin was calculated by 100% - w/w% of water from Karl Fisher analysis.

Example 6: Purification of ADC Mixtures Having Average DARs of 2.7, 4 and 5.5

The following example describes batch purification of several different ADC mixtures comprising either Antibody 1-vc-MMAE or Antibody 1-mc-MMAF having either an average DAR of 2.7 or a more heavily loaded average DAR of 5.5. Additionally, an ADC mixture comprising Antibody 1-vcMMAE with an average DAR of 4 is also described. More specifically, a screen was performed to determine the impact of the resin weight, NaCl concentration, on the purification process (DAR, protein concentration) of two differentially loaded ADCs (antibody 1-vc-MMAE and antibody 1-mc-MMAF) with varying amounts of 6 and 8 loaded species as inputs in the purification process. A series of reaction conditions were tested as described below.

The five crude ADC mixtures (1-5) that were used in Example 6 were prepared as described below. Four crude ADC mixtures (1) Antibody 1-vcMMAE DAR 2.7 (avg), (2) Antibody 1-mcMMAF DAR 2.7 (avg), (3) Antibody 1-vcMMAE DAR 5.5 (avg), and (4) Antibody 1-mcMMAF DAR 5.5 (avg) were prepared in accordance with the methods described in Example 1 and Figure 1 where the reduction of antibody 1 was achieved using TCEP (1.3 or 2.65 molar equivalents) and conjugation was achieved using and of either mc-MMAF or vcMMAE (3 or 6 equivalents) resulting in the preparation of antibody-1-vcMMAE DAR 2.7 (avg) and 5.5 (avg) ADC mixtures and antibody-1-mcMMAF DAR 2.7 (avg) and 5.5 (avg) ADC mixtures. Additionally, a fifth crude ADC mixture (5) Antibody 1-vcMMAE DAR 4 (avg) prepared in accordance of Example 1.

The screening procedure was performed according to the following protocol. First, a respective amount of wet Bu-HIC Resin (representatively prepared as described in Example 2 in the Materials and Method Section) was weighed into a 4 mL vial. The amount of resin was based on a few calculations. First, the amount of dry resin needed was based on the mass amount of 6 and 8 loaded species. The mass amount was calculated based on the crude ADC starting material solution as follows:

Mass amount of species to remove:

Σ(6 load + 8 load), mg = 20 mg of crude ADC (1-5) x (Σ(6 load pa% + 8 load pa%))/100

For example for Vial 13:

Σ(6 load + 8 load), mg = 20 mg of mAb x (10.7/100) = 2.14 mg Σ(6 load + 8 load)

Next, the total mass amount of 6 load and 8 load was multiplied by the target weight of resin. For example for Vial 13:

5 weights of resin = 5 x 2.14 mg Σ(6 load + 8 load) = 10.7 mg dry resin

7.5 weights of resin = 7.5 x 2.14 mg Σ(6 load + 8 load) = 16.1 mg dry resin

10 weights of resin = 10 x 2.14 mg Σ(6 load + 8 load) = 21.4 mg dry resin

Lastly, the resin was corrected for water, sodium chloride, and K_2HPO_4 content. The inorganic salt correction was made because the resin was previously isolated by filtration and washed with 1.95M NaCl/0.05M K_2HPO_4 solution. (The Bu-HIC resin was filtered and washed multiple times with 1.95M NaCl/0.05M K_2HPO_4 solution. The moisture content of the resin was determined by KF (Karl Fisher moisture titration) analysis at 59.0 w/w%. The w/w % concentration of the wash components (10.6 w/w% NaCl, 0.8w/w % K_2HPO_4, 88.6% Water) was then used to estimate the masses of NaCl and K_2HPO_4 in the wet resin (51.8g wet resin, 30.6g water, 3.6g NaCl, and 0.3g K_2HPO_4) which were then subtracted to calculate the dry resin amount (17.3g).)

For example for Vial 13 at 5 weights of dry resin:

10.7 mg dry resin / (0.334 mg dry resin/ mg wet resin) = 32.0 mg wet resin

Following the addition of the Bu-HIC resin, the crude ADC mixtures (1-5) (1.1 – 1.2 mL, 20 mg) was charged into a 4 mL vial. The volume of crude ADC solution was adjusted to target 20mg of total protein.

Next, a range of sodium chloride solutions was prepared. 0.55 – 0.6 mL, (approximately ½ the volume of ADC solution), of the respective molarity of sodium chloride solution/50mM K_2PO_4/pH 7 was charged into various vials. The initial concentration of NaCl solutions were 0 M, 1.95 M, 3.9 M, and 5.85 M at constant concentration of 50mM K2HPO4 at pH 7. After addition to the ADC solutions, the NaCl concentration was reduced to 0, 0.65, 1.3 and 1.95 M NaCl on account of the dilution. The

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vial contents (ADC of DAR 2.5 or 5.5 + salt solution at various concentrations) were then shaken overnight (approximately 20 hrs)

Following stirring overnight (>20 hours), a supernatant sample was taken (remove 0.6 mL through syringe filter, dilute 75 µL of this supernatant sample with 924 µL 1.95N NaCl / 0.05 M K_2HPO_4 pH 7 buffer). Subsequent HPLC-HIC analysis was performed to determine the PA% of each loaded species, as well as protein recovery. The results of the study are shown below in Tables 9 and 10.

Table 9: Summary of Distribution as Measured by HIC-HPLC of vc-MMAE[1]

Vial	Input Σ(6L+8L), pa%	Salt Concentration, Molarity of NaCl	Resin load vs Σ(6L+8L)	0 Load pa%	2 Load pa%	4 Load pa%	6 Load pa%	8 Load pa%	DAR-Overall	%Protein Recovery-Overall*
13	10.7	0	5	16.4	51.6	30.9	1.0	0.0	2.35	106
14	10.7	0	7.5	16.9	53.5	29.2	0.4	0.0	2.28	102
15	10.7	0	10	17.8	55.7	26.2	0.2	0.0	2.20	97
16	10.7	0.65	5	16.9	52.5	29.5	1.0	0.0	2.32	104
17	10.7	0.65	7.5	18.0	55.0	26.8	0.2	0.0	2.20	97
18	10.7	0.65	10	18.8	57.8	22.6	0.3	0.4	2.13	93

[1] Odd species were not included

Vial	Input Σ(6L+8L), pa%	Salt Concentration, Molarity of NaCl	Resin load vs Σ(6L+8L)	0 Load pa%	2 Load pa%	4 Load pa%	6 Load pa%	8 Load pa%	DAR-Overall	%Protein Recovery-Overall*
19	10.7	1.3	5	17.5	52.9	27.9	0.6	1.0	2.30	100
20	10.7	1.3	7.5	20.2	55.2	23.3	0.6	0.7	2.13	86
21	10.7	1.3	10	20.0	57.9	21.2	0.4	0.5	2.08	88
22	10.7	1.95	5	25.2	52.2	18.6	1.5	2.5	2.08	69
23	10.7	1.95	7.5	19.2	55.1	24.8	0.3	0.6	2.17	92
24	10.7	1.95	10	21.9	58.2	18.8	0.5	0.5	2.00	77
37	60.2	0	5	1.4	26.8	70.5	1.3	0.0	3.41	39
38	60.2	0	7.5	2.9	52.2	41.2	1.2	2.4	2.94	21
39	60.2	0	10	6.1	89.0	4.9	0.0	0.0	2.00	10
40	60.2	0.65	5	2.0	30.7	65.8	0.8	0.7	3.30	31
41	60.2	0.65	7.5	6.9	87.7	5.4	0.0	0.0	1.98	9
42	60.2	0.65	10	21.8	78.2	0.0	0.0	0.0	1.47	3
43	60.2	1.3	5	7.5	89.7	1.0	1.8	0.0	2.60	21
44	60.2	1.3	7.5	44.3	55.7	0.0	0.0	0.0	1.09	1
45	60.2	1.3	10	100.0	0.0	0.0	0.0	0.0	0.00	0
46	60.2	1.95	5	1.9	28.3	68.4	0.7	0.7	3.37	20
47	60.2	1.95	7.5	100.0	0.0	0.0	0.0	0.0	0.00	0
48	60.2	1.95	10	0.00	0.00	0.00	0.00	0.00	0.00	0
49	25.9	0	5	7.0	44.4	47.0	1.6	0.0	2.91	88
50	25.9	0	7.5	8.1	50.6	41.3	0.0	0.0	2.68	75
51	25.9	0	10	9.3	58.2	32.5	0.0	0.0	2.49	66
52	25.9	0.65	5	7.5	46.2	45.9	0.3	0.0	2.81	82
53	25.9	0.65	7.5	8.9	53.8	37.3	0.0	0.0	2.59	69

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Vial	Input Σ(6L+8L), pa%	Salt Concentration, Molarity of NaCl	Resin load vs Σ(6L+8L)	0 Load pa%	2 Load pa%	4 Load pa%	6 Load pa%	8 Load pa%	DAR-Overall	%Protein Recovery-Overall*
54	25.9	0.65	10	11.4	66.6	22.0	0.0	0.0	2.24	55
55	25.9	1.3	5	8.1	47.0	44.3	0.5	0.0	2.76	76
56	25.9	1.3	7.5	9.9	54.5	35.6	0.0	0.0	2.53	63
57	25.9	1.3	10	14.3	68.8	16.9	0.0	0.0	2.07	43
58	25.9	1.95	5	8.3	46.9	43.9	0.8	0.0	2.77	74
59	25.9	1.95	7.5	10.2	55.1	34.7	0.0	0.0	2.50	58
60	25.9	1.95	10	14.2	72.1	13.7	0.0	0.0	2.02	40

* Protein recovery was calculated using UV by HPLC at 280 nm.

Table 10: Summary of Distribution as Measured by HIC-HPLC of mc-MMAF[2]

Vial	Input Σ(6L+8L), pa%	Salt Concentration, Molarity of NaCl	Resin load vs Σ(6L+8L)	0 Load pa% @280nm	2 Load pa% @280nm	4 Load pa% @280nm	6 Load pa% @280nm	8 Load pa% @280nm	DAR-Overall	%Protein Recovery-Overall*
1	9.4	0	5	17.8	37.2	31.3	6.7	0.9	2.64	97
2	9.4	0	7.5	17.8	37.8	31.6	6.0	0.6	2.61	97
3	9.4	0	10	18.1	38.3	31.4	5.4	0.2	2.55	95

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Vial	Input Σ(6L+8L), pa%	Salt Concentration, Molarity of NaCl	Resin load vs Σ(6L+8L)	0 Load pa% @280nm	2 Load pa% @280nm	4 Load pa% @280nm	6 Load pa% @280nm	8 Load pa% @280nm	DAR-Overall	%Protein Recovery-Overall*
4	9.4	0.65	5	18.1	39.2	32.4	5.1	0.2	2.55	97
5	9.4	0.65	7.5	18.5	39.8	32.2	3.6	0.4	2.49	95
6	9.4	0.65	10	18.6	40.0	32.2	3.0	0.5	2.47	95
7	9.4	1.3	5	19.4	42.3	33.9	4.5	0.3	2.50	95
8	9.4	1.3	7.5	19.7	42.3	30.3	2.6	0.3	2.37	90
9	9.4	1.3	10	20.5	45.3	28.9	1.8	0.2	2.31	86
10	9.4	1.95	5	19.0	40.8	31.6	3.8	0.2	2.45	95
11	9.4	1.95	7.5	19.9	42.7	30.8	1.9	0.1	2.33	89
12	9.4	1.95	10	21.2	45.2	28.1	1.2	0.1	2.21	82
25	56.8	0	5	1.7	9.6	36.3	29.3	14.1	4.93	84
26	56.8	0	7.5	0.8	10.8	41.8	27.4	9.5	4.69	72
27	56.8	0	10	0.8	11.9	46.1	26.9	4.4	4.45	61
28	56.8	0.65	5	0.9	12.0	45.4	25.3	4.7	4.43	67
29	56.8	0.65	7.5	1.0	16.5	59.4	12.6	0.5	3.89	45
30	56.8	0.65	10	1.6	20.1	64.1	5.3	0.0	3.59	35
31	56.8	1.3	5	1.0	15.4	54.5	16.7	2.2	4.08	51
32	56.8	1.3	7.5	1.6	22.8	64.7	2.9	0.0	3.48	30
33	56.8	1.3	10	3.2	36.2	51.8	0.0	0.0	3.01	18
34	56.8	1.95	5	1.5	18.9	57.0	9.5	3.6	3.88	38
35	56.8	1.95	7.5	3.0	33.1	57.2	0.0	0.0	3.11	18
36	56.8	1.95	10	7.9	58.1	22.6	0.0	0.0	2.29	7

* Protein recovery was calculated using UV by HPLC at 280 nm. [2] Odd species were not included.

In sum, the resin titration screen described in Example 6, Tables 9 and 10 was performed to determine the impact of the resin load and NaCl concentration on the purification process (DAR, protein concentration) obtained from the crude Antibody-1-mcMMAF and Antibody-1-vcMMAE ADC mixtures (1) - (5) possessing a DAR (avg) range of 2.7 to 5.5. The calculated amount of resin as shown in Tables 9 and 10, depends on the drug load species to be removed from the crude distribution. For example, a resin weight of approximately 5 to 10 times that of the 6 and 8 drug load species was proven to be effective for removing these species from the crude reaction mixture.

Example 7: Batch and Flow Through Purification of Antibody 1-vc-MMAE

Batch Purification Method

Generation of a crude distribution of an ADC, *i.e.*, Antibody 1-vc-MMAE, was performed in accordance with the methods described in Example 1.

The reaction mixture was then treated with Bu-HIC resin that was previously washed with 50 mM potassium phosphate, 2 M NaCl, and buffer at a pH of 6.8. The resin / reaction mixture was subsequently stirred, and monitored by analytical hydrophobic interaction chromatography for removal of drug conjugate products (according to the methods described in the previous examples). Results of this additional experiment describing batch purification of Antibody 1-vc-MMAE are described in Table 11. Retention time referred to in Table 11 is the time for which the compound takes to elute off of the analytical HPLC analysis.

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Table 11: Peak Area % Results (PA%) of the Reaction Mixture Before (Labeled "Reaction" Below) and After Purification (Labeled "Purified" Below)

HIC pa% results @ 280nm			
Retention time (min)	DAR	Reaction	Purified
6.4	0	5.15	6.54
7.2	1	0.36	1.46
8.4	2	27.26	33.37
10.2	4	40.74	50.54
11.3	5	6.03	5.86
11.8	6	11.45	1.67
12.2	7	1.67	0.55
12.4	7	2.00	
12.7	8	5.32	
DAR(avg) =		3.9	3.1
	E4/E2 =	1.5	1.5
Σ (>E6), pa% 280nm =		20.4	2.2

As described in Table 11, batch purification of Antibody-1-vc-MMAE resulted in a lower average DAR relative to the initial reaction mixture.

Flow Through Purification Method

Alternatively, the purification of Antibody 1-vc-MMAE may be performed using a flow through purification mode.

Flow through purification was generally performed according to the following method: A two liter batch of Tosoh Bioscience Butyl 600 M resin was made. Resin was filtered into a 2 L sintered funnel (note the funnel had been previously washed with IPA and dried). Filtered resin was washed with 2 x 2 L of 50 mM potassium phosphate, 2 M NaCl phosphate buffer at pH 6.8. The resin potency was determined to be 27% by Karl Fisher moisture analysis (analysis showed the presence of 73 w/w% water; note in this example the modest amount of inorganic residue (NaCl and K_2HPO_4) was not used in calculating the potency of the resin).

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Reduction / conjugation methods described in Example 1 were used, starting with 134.9 g of Antibody 1. One change relative to the protocol described in Example 1 was that 2.15 equiv TCEP was used (which resulted in a slightly higher average DAR). The process resulted in 6-8 load species that were 33.8 pa%. Thus 45.6 g of 6-8 load species was present in the crude reaction mixture.

Using a 5x loading of hydrophobic resin, 228.5 g dry weight hydrophobic resin was required (*i.e.* 45.6 g of 6-8 load species multiplied by 5 = 228.5 g of potency adjusted resin). At a resin potency of 27% (note: potency of resin is calculated by 100% - w/w% of water from Karl Fisher analysis), 856 g wet weight resin equivalent to 228.5 grams of potency adjusted resin (i.e. calculation: 228.5 grams of potency adjusted resin / (27 grams dry-weight resin/100 grams wet resin)) = 856 g wet resin required) was loaded into a stainless steel column that was 4 inches x 7 inches. The crude reaction mixture was then pumped from a sterile 20 L carboy over the resin bed through a pressure sensor and a peristaltic pump using size 35 Pharmed tubing and into a second 20 L sterile carboy at 185 ml/min. The collected filtrate was then pumped across the resin bed again, collecting the desired ADC mixture containing the lower DAR species in the final filtrate. The flow through process used was a double pass process.

The resin bed was then washed numerous times to remove residual unbound lower DAR species while leaving the high DAR species (drug loads 6-8) bound to the resin. Specifically, first, the resin bed was washed with 1200 mL 1 N NaCl (95 mS) prepared by diluting 600 ml of the 50 mM potassium phosphate, 2 M NaCl to 1200ml with WFI. The resin bed was then washed with 1200 ml 0.75 N NaCl (71 mS) prepared by diluting 450 ml of the 50 mM potassium phosphate, 2 M NaCl to 1200 mL with WFI. A third wash was performed using 1200 mL 0.5 N NaCl (50 mS) prepared by diluting 300 ml of the 50 mM potassium phosphate, 2 M NaCl with 900 ml WFI. A fourth wash was performed using 1200 mL 0.25 N NaCl (26 mS) prepared by diluting 150 ml of the 50 mM potassium phosphate, 2 M NaCl to 1200 ml with WFI. The filtrate from resin washes was largely collected and combined with the final filtrate from the above flow through process, affording the bulk (*i.e.*, final filtrate + washes).

Notably, the washing of the resin bed is optional, as purified ADCs having a DAR of 2-4 were obtained in the final filtrate from the initial multi-pass procedure described above.

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The first wash provided about 10% recovery from the wash, while the subsequent washes resulted in about 1-2% recovery.

The bulk was concentrated by tangential flow filtration (TFF) to approximately 1200 g of concentrated ADC solution and then exchanged with 10 diavolumes of 15 mM Histidine buffer at pH 6 to yield the desired DAR 0 – 4 species of Antibody 1-vc-MMAE at a final protein concentration of 35 mg/mL (isolated 81 grams, 66% yield, 91% recovery vs DAR 0-4). Thus, the flow through purification method was successful at separating DAR species, *i.e.*, high load species of 6-8, away from the lower DAR species (described in more detail below in Table 12).

A comparison of the batch purification mode to the flow through purification mode (Table 12) was performed. In both cases, the loading of resin vs. protein was 5 weights of resin vs. the mass of the high load DAR 6-8 species of Antibody 1-vc-MMAE. Although there was a slight variance of the relative amount of the individual species (due to the slightly higher equivalents of TCEP used in the flow through experiment example), the effectiveness of removing the higher DAR species of both methods was comparable. The material referred to in Table 12 in the "Flow Through Method" column includes the combined filtrate from the flow through method and the material from the washes (referred to collectively as "bulk").

Table 12. Comparison of Batch vs Flow-Through Methods

HIC PA% results @ 280 nm			
Retention time, (min)	DAR	Purified (Batch)	Purified (Flow Through Method)
4.3	0	10.7	7.9
5.9	2	46.2	41.6
7.5	4	41.7	47.0
8.8	6	1.4	3.5
9.9	8	Not detected	Not detected
	DAR =	2.7	2.9

In conclusion, both the batch and flow through purification methods were used to enrich for ADCs having DARs of 2-4. Both purification methods relied on the ratio of protein (ADC) weight (coupled with fraction of high drug load species) to the load of resin

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used, where a hydrophobic resin weight which is 5 to 6 times the weight of the drug loaded species of 6-8 (6 or more) in the ADC mixture resulted in substantially reduced levels of ADCs having a DAR of 6-8. As described in Table 12, both processes resulted in compositions comprising at least 95% ADCs having a DAR of 4 or less or compositions comprising ADCs with less than 4% of the drug loaded species or 6 or more.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

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What is claimed is:

1. A method of obtaining a composition comprising Antibody Drug Conjugates (ADCs), said method comprising

contacting an ADC mixture comprising a drug loaded species of 4 or less and a drug loaded species of 6 or more with a hydrophobic resin, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug loaded species of 4 or less; and

removing the hydrophobic resin from the ADC mixture, such that the composition comprising ADCs is obtained,

wherein the composition comprises less than 15% of the drug loaded species of 6 or more, and

wherein the ADC comprises an antibody conjugated to an auristatin.

2. The method of claim 1, wherein the composition comprises less than 10% of the drug loaded species of 6 or more.

3. The method of claim 1, wherein the composition comprises 5% or less of the drug loaded species of 6 or more.

4. The method of any one of claims 1-3, wherein the hydrophobic resin weight is 3 to 12 times the weight of the drug loaded species of 6 or more in the ADC mixture.

5. The method of any one of claims 1-3, wherein the hydrophobic resin weight is 4 to 8 times the weight of the drug loaded species of 6 or more in the ADC mixture.

6. The method of any one of claims 1-3, wherein the hydrophobic resin weight is 5 to 10 times the weight of the drug loaded species of 6 or more in the ADC mixture.

7. The method of any one of claims 1-3, wherein the hydrophobic resin weight is 6 to 12 times the weight of the drug loaded species of 6 or more in the ADC mixture, and wherein the ADC mixture comprises between 0 to 1 N NaCl, or an equivalent ionic strength thereof.

8. The method of claim 4, wherein the hydrophobic resin weight is 3 to 6 times the weight of the drug loaded species of 6 or more in the ADC mixture, and wherein the ADC mixture comprises between 1 to 2N NaCl, or an equivalent ionic strength thereof.

9. The method of any one of claims 1-3, wherein the hydrophobic resin weight is 3 to 7 times the weight of the drug loaded species of 6 or more in the ADC mixture, and wherein the auristatin is monomethylauristatin E (MMAE).

10. The method of any one of claims 1-3, wherein the hydrophobic resin weight is 5 to 10 times the weight of the drug loaded species of 6 or more in the ADC mixture, and wherein the auristatin is monomethylauristatin F (MMAF).

11. The method of any one of claims 1-3, wherein the hydrophobic resin weight is 3 to 7 times the weight of the drug loaded species of 6 or more in the ADC mixture, and wherein the auristatin is monomethylauristatin E (MMAE).

12. A method of producing a composition comprising ADCs with an average Drug-to-Antibody Ratio (DAR) of 4.5 or less and comprising less than 15% undesired ADCs, said method comprising

contacting an ADC mixture with a hydrophobic resin, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the undesired ADCs; and

removing the hydrophobic resin from the ADC mixture, such that the composition with an average DAR of 4.5 or less and comprising less than 15% undesired ADCs is produced,

wherein the ADC comprises an antibody conjugated to an auristatin.

13. The method of claim 12, wherein the composition with an average DAR of 4.5 or less comprises less than 10% undesired ADCs.

14. The method of claim 13, wherein the undesired ADCs are 6 and 8 drug loaded species.

15. The method of any one of claims 12-14, wherein the amount of hydrophobic resin added to the ADC mixture is a resin weight which is 3 to 12 times the weight of the undesired ADCs in the ADC mixture.

16. The method of any one of claims 12-14, wherein the amount of hydrophobic resin added to the ADC mixture is a resin weight which is 4 to 8 times the weight of the drug loaded species of 6 or more in the ADC mixture.

17. The method of any one of claims 12-14, wherein the amount of hydrophobic resin added to the ADC mixture is a resin weight which is 5 to 7 times the weight of the drug loaded species of 6 or more in the ADC mixture.

18. The method of any one of claims 12-14, wherein the hydrophobic resin weight is 6 to 12 times the weight of the drug loaded species of 6 or more in the ADC mixture and wherein the ADC mixture comprises between 0 to 1 N NaCl, or an equivalent ionic strength thereof.

19. The method of any one of claims 12-14, wherein the hydrophobic resin weight is 3 to 6 times the weight of the drug loaded species of 6 or more in the ADC mixture and wherein the ADC mixture comprises between 1 to 2 N NaCl, or an equivalent ionic strength thereof.

20. The method of any one of claims 12-14, wherein the hydrophobic resin weight is 3 to 7 times the weight of the drug loaded species of 6 or more in the ADC mixture, and wherein the auristatin is monomethylauristatin E (MMAE).

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21. The method of any one of claims 12-14, wherein the hydrophobic resin weight is 5 to 10 times the weight of the drug loaded species of 6 or more in the ADC mixture, and wherein the auristatin is monomethylauristatin F (MMAF).

22. The method of any one of claims 12-14, wherein the hydrophobic resin weight is 3 to 7 times the weight of the drug loaded species of 6 or more in the ADC mixture, and wherein the auristatin is monomethylauristatin E (MMAE).

23. The method of any one of claims 13-23, wherein the composition has an average DAR of 4 or less.

24. The method of any one of claims 13-23, wherein the composition has an average DAR of 3.5 or less.

25. The method of any one of claims 13-23, wherein the composition has an average DAR of 3 or less.

26. The method of any one of claims 13-23, wherein the composition has an average DAR of 2.5 or less.

27. The method of any one of claims 1-26, wherein the ADC mixture was obtained following an ultrafiltration /diafiltration process.

28. The method of any one of claims 1-27, wherein the hydrophobic resin is a butyl hydrophobic resin.

29. The method of any one of claims 1-28, which is a batch process, a circulation process or a flow through process.

30. The method of any one of claims 1-29, wherein the ADC comprises an anti-Epidermal Growth Factor Receptor (EGFR) antibody.

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31. The method of claim 30, wherein the anti-EGFR antibody comprises a light chain variable region comprising a Complementarity Determining Region 1 (CDR1), CDR2, and CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9, respectively, and comprises a heavy chain variable region comprising a CDR1, CDR2, and CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4.

32. The method of claim 30, wherein the anti-EGFR antibody comprises a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 6 and a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 1.

33. The method of any one of claims 1-32, wherein the auristatin is either monomethylauristatin E (MMAE) or monomethylauristatin F (MMAF).

34. The method of claim 33, wherein the MMAE is conjugated to the antibody via a valine-citrulline (vc) linker.

35. The method of claim 33, wherein the MMAF is conjugated to the antibody via a malemidocaproyl (mc) linker.

36. A composition obtained through any one of the methods of claims 1-35.

37. A method of treating cancer in a subject comprising administering the composition of claim 36 to the subject such that cancer is treated.

38. A composition comprising ADCs, wherein 70% of ADCs present have a drug loaded species of 4 or less, and wherein the ADC comprises an anti-EGFR antibody and an auristatin.

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39. The composition of claim 38, wherein 75% of ADCs present have a drug loaded species of 4 or less.

40. The composition of claim 38, wherein 80% of ADCs present have a drug loaded species of 4 or less.

41. The composition of claim 38, wherein 85% of ADCs present have a drug loaded species of 4 or less.

42. The composition of claim 38, wherein 90% of ADCs present have a drug loaded species of 4 or less.

43. The composition of claim 38, wherein 95% of ADCs present have a drug loaded species of 4 or less.

44. The composition of any one of claims 38-43, wherein the anti-EGFR antibody comprises a light chain variable region comprising a Complementarity Determining Region 1 (CDR1), CDR2, and CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9, respectively, and comprises a heavy chain variable region comprising a CDR1, CDR2, and CDR3 domain comprising the amino acid sequence as set forth in SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4.

45. The composition of any one of claims 38-43, wherein the anti-EGFR antibody comprises a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 6 and a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 1.

46. The composition of any one of claims 38-45, wherein the auristatin is either monomethylauristatin E (MMAE) or monomethylauristatin F (MMAF).

47. The composition of claim 46, wherein the MMAE is conjugated to the antibody via a valine-citrulline (vc) linker..

48. The composition of claim 46, wherein the MMAF is conjugated to the antibody via a malemidocaproyl (mc) linker.

49. A pharmaceutical composition comprising the composition of any one of claims 38-48 and a pharmaceutically acceptable carrier.

50. A method of treating cancer in a subject comprising administering the pharmaceutical composition of claim 49 to the subject, such that cancer is treated.

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Figure 1

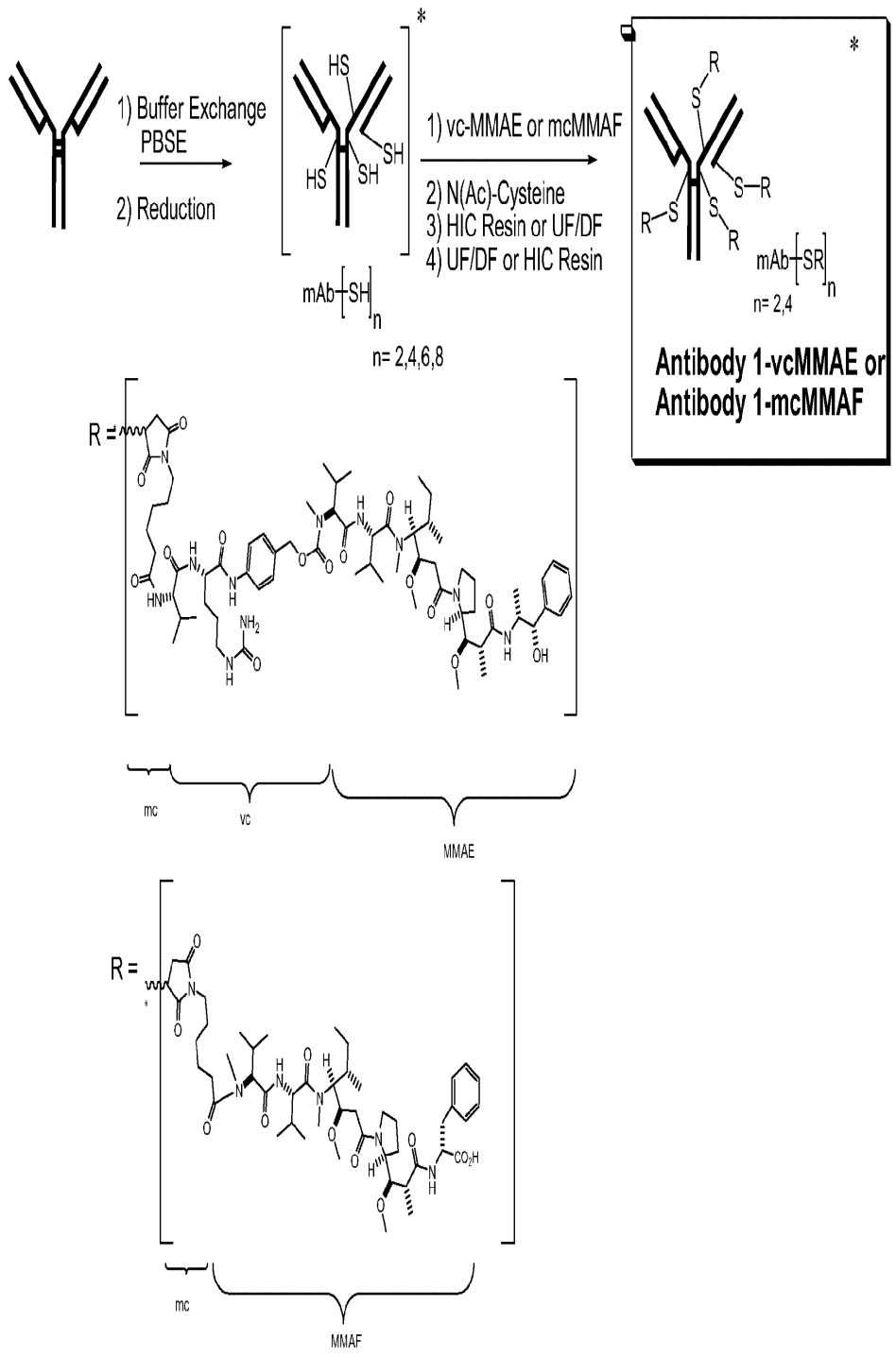

1) Buffer Exchange
PBSE
2) Reduction
1) vc-MMAE or mcMMAF
2) N(Ac)-Cysteine
3) HIC Resin or UF/DF
4) UF/DF or HIC Resin
mAb-[SH]n
n= 2,4,6,8
mAb-[SR]n
n= 2,4
Antibody 1-vcMMAE or
Antibody 1-mcMMAF
R =
mc
vc
MMAE
R =
mc
MMAF

Figure 2

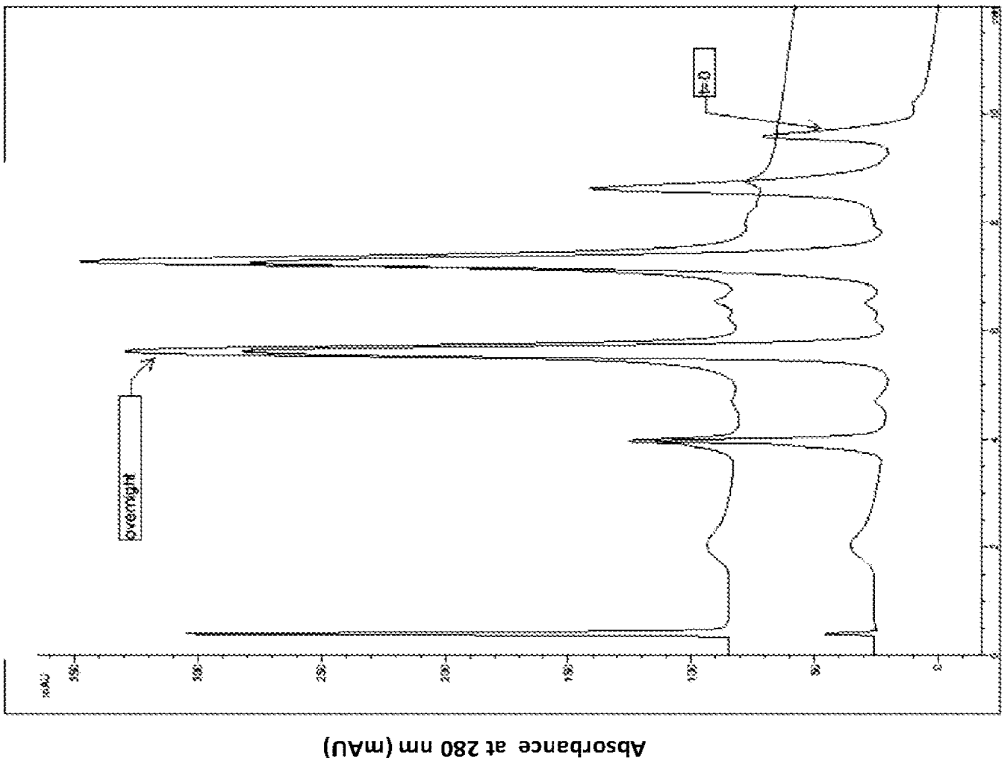
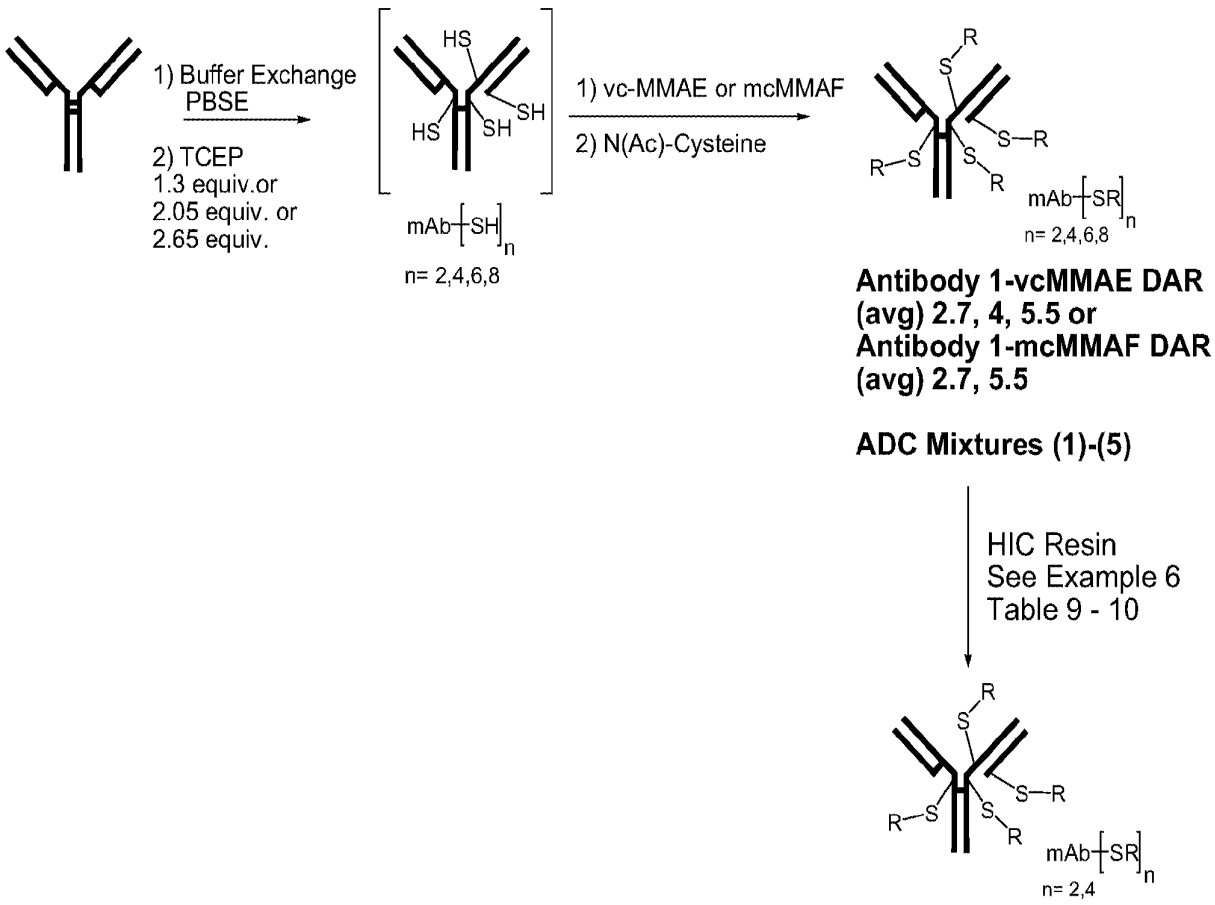


Figure 3


1) Buffer Exchange
PBSE
2) TCEP
1.3 equiv.or
2.05 equiv. or
2.65 equiv.
HS
HS
SH
SH
mAb-[SH]n
n= 2,4,6,8
1) vc-MMAE or mcMMAF
2) N(Ac)-Cysteine
R
S
S—R
R—S
S
R
mAb-[SR]n
n= 2,4,6,8
Antibody 1-vcMMAE DAR (avg) 2.7, 4, 5.5 or
Antibody 1-mcMMAF DAR (avg) 2.7, 5.5
ADC Mixtures (1)-(5)
HIC Resin
See Example 6
Table 9 - 10
mAb-[SR]n
n= 2,4

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/027062

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K47/48 A61P35/00
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)

EPO-Internal, WPI Data, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KEVIN J HAMBLETT ET AL: "Effects of drug loading on the antitumor activity of a monoclonal antibody drug conjugate", CLINICAL CANCER RESEARCH, THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 10, no. 20, 15 October 2004 (2004-10-15), pages 7063-7070, XP002623986, ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-04-0789 page 7064, column 1, paragraph 4 - column 2, paragraph 2 page 7065, column 2, paragraph 5 - page 7066, column 1, paragraph 1 figures 1B, 1C ----- -/--	1-9, 11-20, 22-27, 29,33, 34,36,37

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 July 2014	01/08/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Monami, Amélie

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JEAN-PHILIPPE STEPHAN ET AL: "Anti-CD22-MCC-DM1 and MC-MMAF Conjugates: Impact of Assay Format on Pharmacokinetic Parameters Determination", BIOCONJUGATE CHEMISTRY, vol. 19, no. 8, 1 August 2008 (2008-08-01) , pages 1673-1683, XP055067530, ISSN: 1043-1802, DOI: 10.1021/bc800059t table 1 page 1674, column 1, paragraph 2 - page 1675, column 1, paragraph 1 figure 3 page 1679, column 2, paragraph 3	1-8,10, 12-19, 21, 23-27, 29,33, 35-37
X,P	WO 2013/163631 A2 (CYTOMX THERAPEUTICS INC [US]) 31 October 2013 (2013-10-31) examples 9, 10	38-50
A	WO 2011/041319 A2 (LUDWIG INST CANCER RES [US]; OLD LLOYD J [US]; JOHNS TERRANCE GRANT [A) 7 April 2011 (2011-04-07) cited in the application examples 22, 25 figures 55A, 55B	1-50

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2014/027062

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013163631 A2	31-10-2013	US 2013315906 A1	28-11-2013
		WO 2013163631 A2	31-10-2013
WO 2011041319 A2	07-04-2011	AR 080663 A1	02-05-2012
		TW 201124155 A	16-07-2011
		US 2011076232 A1	31-03-2011
		UY 32915 A	29-04-2011
		WO 2011041319 A2	07-04-2011

Espacenet

Bibliographic data: CN103772504 (A) — 2014-05-07

Humanized antibody against epidermal growth factor receptor and application thereof

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Classification: **- international:** ***A61K39/395; A61K48/00; A61P35/00; C07K16/28; C12N1/15; C12N1/19; C12N1/21; C12N15/13; C12N15/63; C12N5/10; G01N33/574; G01N33/68***

- cooperative:

Application number: CN20121406288 20121017 Global Dossier

Priority number (s): CN20121406288 20121017

Also published as: CN103772504 (B)

Abstract of CN103772504 (A)

The invention relates to a humanized antibody against an epidermal growth factor receptor. The invention further relates to a nucleic acid molecule coding the antibody, a vector, a host cell and a composition containing the nucleic acid molecule, a detection reagent and a kit. The invention also relates to application of the antibody in preparation of drugs used for prevention/treatment of diseases related to an epidermal growth factor or the epidermal growth factor acceptor and preparation of the detection reagent or kit. The molecule of the antibody provided by the invention has lower immunogenicity and higher target binding activity, so the antibody has stronger biological activity and in-vitro and in-vivo antineoplastic activity, and it is expected that the antibody has a smaller side-effect and a better curative effect in clinic application.

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C12N 5/10(2006.01)
A61K 39/395(2006.01)
A61K 48/00(2006.01)
A61P 35/00(2006.01)
G01N 33/68(2006.01)
G01N 33/574(2006.01)

权利要求书2页 说明书15页
序列表13页 附图3页

(54)发明名称

人源化抗表皮生长因子受体抗体及其用途

(57)摘要

本发明涉及人源化抗表皮生长因子受体抗体。本发明还涉及编码所述抗体的核酸分子，含有所述核酸分子的载体、宿主细胞和组合物，以及检测试剂和试剂盒。本发明还涉及所述抗体用于制备预防/治疗表皮生长因子或表皮生长因子受体相关疾病的药物中的用途以及用于制备检测试剂或试剂盒中的用途。本发明的抗体分子具有更低的免疫原性和更高的的靶点结合活性，从而有更强的生物学活性和体内外抗肿瘤活性，可以预期临床应用副作用更低，疗效更好。

CN 103772504 A

1. 抗表皮生长因子受体抗体或其抗原结合部分，其重链CDR1的序列选自SEQ ID NO:1和SEQ ID NO:39所示序列或其衍生序列，CDR3的序列选自SEQ ID NO:2、SEQ ID NO:3和SEQ ID NO:40所示序列或其衍生序列；和/或

其轻链CDR3的序列选自SEQ ID NO:9、SEQ ID NO:10和SEQ ID NO:41所示序列或其衍生序列。

2. 权利要求1的抗体或其抗原结合部分，其重链可变区CDR的序列选自以下(1)～(4)所示序列或其衍生序列：

(1)CDR1的序列为SEQ ID NO:1所示序列，CDR2的序列为SEQ ID NO:4所示序列，CDR3的序列为SEQ ID NO:2所示序列；

(2)CDR1的序列为SEQ ID NO:1所示序列，CDR2的序列为SEQ ID NO:4所示序列，CDR3的序列为SEQ ID NO:40所示序列；

(3)CDR1的序列为SEQ ID NO:39所示序列，CDR2的序列为SEQ ID NO:4所示序列，CDR3的序列为SEQ ID NO:3所示序列；

(4)CDR1的序列为SEQ ID NO:39所示序列，CDR2的序列为SEQ ID NO:4所示序列，CDR3的序列为SEQ ID NO:40所示序列；和/或

其轻链可变区CDR的序列选自以下(5)～(8)所示序列或其衍生序列：

(5)CDR1的序列为SEQ ID NO:11所示序列，CDR2的序列为SEQ ID NO:12所示序列，CDR3的序列为SEQ ID NO:9所示序列；

(6)CDR1的序列为SEQ ID NO:11所示序列，CDR2的序列为SEQ ID NO:12所示序列，CDR3的序列为SEQ ID NO:41所示序列；

(7)CDR1的序列为SEQ ID NO:11所示序列，CDR2的序列为SEQ ID NO:12所示序列，CDR3的序列为SEQ ID NO:41所示序列；

(8)CDR1的序列为SEQ ID NO:11所示序列，CDR2的序列为SEQ ID NO:12所示序列，CDR3的序列为SEQ ID NO:10所示序列。

3. 权利要求1或2的抗体或其抗原结合部分，其重链FR1的序列选自SEQ ID NO:19～SEQ ID NO:22所示序列或其衍生序列，FR2的序列选自SEQ ID NO:23～SEQ ID NO:25所示序列或其衍生序列，FR3的序列选自SEQ ID NO:26～SEQ ID NO:29所示序列或其衍生序列，FR4的序列为SEQ ID NO:30所示序列或其衍生序列；和/或

其轻链FR1的序列选自SEQ ID NO:31～SEQ ID NO:32所示序列或其衍生序列，FR2的序列选自SEQ ID NO:33～SEQ ID NO:34所示序列或其衍生序列，FR3的序列选自SEQ ID NO:35～SEQ ID NO:36所示序列或其衍生序列，FR4的序列选自SEQ ID NO:37～SEQ ID NO:38所示序列或其衍生序列。

4. 权利要求3的抗体或其抗原结合部分，其重链可变区FR的序列选自以下(9)～(12)所示序列或其衍生序列：

(9)FR1的序列为SEQ ID NO:19所示序列，FR2的序列为SEQ ID NO:23所示的序列，FR3的序列为SEQ ID NO:26所示的序列，FR4的序列为SEQ ID NO:30所示的序列；

(10)FR1的序列为SEQ ID NO:20所示序列，FR2的序列为SEQ ID NO:24所示的序列，FR3的序列为SEQ ID NO:27所示的序列，FR4的序列为SEQ ID NO:30所示的序列；

(11)FR1的序列为SEQ ID NO:21所示序列，FR2的序列为SEQ ID NO:23所示的序列，

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FR3 的序列为 SEQ ID NO :28 所示的序列，FR4 的序列为 SEQ ID NO :30 所示的序列 ；

(12)FR1 的序列为 SEQ ID NO :22 所示序列，FR2 的序列为 SEQ ID NO :25 所示的序列，FR3 的序列为 SEQ ID NO :29 所示的序列，FR4 的序列为 SEQ ID NO :30 所示的序列 ；和 / 或

其轻链可变区 FR 的序列选自以下 (13) ～ (16) 所示序列或其衍生序列 ：

(13)FR1 的序列为 SEQ ID NO :31 所示序列，FR2 的序列为 SEQ ID NO :33 所示的序列，FR3 的序列为 SEQ ID NO :35 所示的序列，FR4 的序列为 SEQ ID NO :37 所示的序列 ；

(14)FR1 的序列为 SEQ ID NO :31 所示序列，FR2 的序列为 SEQ ID NO :33 所示的序列，FR3 的序列为 SEQ ID NO :35 所示的序列，FR4 的序列为 SEQ ID NO :38 所示的序列 ；

(15)FR1 的序列为 SEQ ID NO :32 所示序列，FR2 的序列为 SEQ ID NO :34 所示的序列，FR3 的序列为 SEQ ID NO :36 所示的序列，FR4 的序列为 SEQ ID NO :38 所示的序列 ；

(16)FR1 的序列为 SEQ ID NO :31 所示序列，FR2 的序列为 SEQ ID NO :33 所示的序列，FR3 的序列为 SEQ ID NO :35 所示的序列，FR4 的序列为 SEQ ID NO :37 所示的序列。

5. 权利要求 1-4 任一项的抗体或其抗原结合部分，其还包含人 IgG1 重链恒定区和人 κ 轻链恒定区。

6. 权利要求 1-4 任一项的抗体或其抗原结合部分，其中所述抗原结合部分为单链抗体 (scFv)、嵌合抗体、双抗体、scFv-Fc 二价分子、dAb 和互补决定区 (CDR) 片段、Fab 片段、Fab′ 片段、Fv 或 $F(ab')_2$ 片段。

7. 分离的核酸分子，其编码权利要求 1-6 任一项所述的抗体或其抗原结合部分。

8. 载体，其含有权利要求 7 的核酸分子。

9. 宿主细胞，其含有权利要求 7 的核酸分子或权利要求 8 的载体。

10. 组合物，其含有权利要求 1-6 任一项的抗体或其抗原结合部分，以及任选的一种或多种药学可接受的辅料 ；优选地，所述辅料选自蛋白水解酶抑制剂、药用载体、稀释剂和佐剂。

11. 权利要求 1-6 任一项的抗体或其抗原结合部分、或权利要求 7 的核酸分子、或权利要求 8 的载体、或权利要求 9 的宿主细胞在制备预防 / 治疗表皮生长因子或表皮生长因子受体相关疾病的药物中的用途 ；优选地，所述表皮生长因子或表皮生长因子受体相关疾病是指由于表皮生长因子高表达或表皮生长因子受体高表达而引起的疾病 ；更优选地，所述由于表皮生长因子高表达或表皮生长因子受体高表达而引起的疾病是指恶性肿瘤。

12. 检测试剂或试剂盒，其含有权利要求 1-6 任一项的抗体或其抗原结合部分 ；

优选地，所述抗体或其抗原结合部分还可包含可检测的标记 ；或者

优选地，所述检测试剂或试剂盒还可包含第二抗体，其特异性识别所述抗体或其抗原结合部分 ；或者

优选地，所述第二抗体还可包含可检测的标记。

13. 权利要求 1-6 任一项的抗体或其抗原结合部分在制备检测试剂或试剂盒中的用途，其中所述检测试剂或试剂盒用于检测表皮生长因子或表皮生长因子受体，或者用于诊断表皮生长因子或表皮生长因子受体相关疾病。

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人源化抗表皮生长因子受体抗体及其用途

技术领域

[0001] 本发明涉及人源化抗体，具体涉及人源化抗表皮生长因子受体抗体，本发明还涉及所述抗体在制备预防或治疗与表皮生长因子高活性或高表达相关疾病的药物中的用途。与人鼠嵌合抗体相比，本发明所涉及的人源化抗表皮生长因子抗体进一步降低了抗体的免疫原性，同时增强了其抗原识别活性和体内体外抗瘤功能，有更好的临床应用前景。

背景技术

[0002] 表皮生长因子受体 (Epidermal Growth Factor Receptor，EGFR，也称为 HER1，c-ErbB1) 是表皮生长因子家族的细胞表面受体，是由 1186 个氨基酸残基构成，分子量为 170kD 的一种跨膜糖蛋白 (Jorissen RN，Walker F，Pouliot N，et al.Epidermal growth factor receptor：mechanisms of activation and signaling.Exp Cell Res，2003；284：31-53)。EGFR 属于 I 型酪氨酸激酶受体 ErbB 亚族 (ErbB 1-4)，具有酪氨酸激酶的活性。EGFR 稳定的表达于许多上皮组织，包括皮肤和毛囊。表皮生长因子受体异常表达或因受体突变而活化会导致癌变。有很多实体瘤发现高表达表皮生长因子受体，如直结肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌和食管癌等 (Olayioye MA，Neve RM，Lane HA，et al.The EerbB Signaling network：receptor heterodimerzation in development and cancer.The EMBO J，2000；19：3159-3167)。转化生长因子 α 和表皮生长因子等生长因子是表皮生长因子受体的内源性配体。这些配体与表皮生长因子受体结合，激活受体胞内酪氨酸蛋白激酶的活性，启动下游多条信号转导途径，从而调节正常细胞的生长和分化，增加肿瘤细胞的侵袭力、促进血管生成、抑制肿瘤细胞的凋亡 (Ciardiello F，Tortora G.A novel approach in the treatment of cancer：targeting the epidermal growth factor receptor.Clin Cancer Res，2001；7：2958-2970)。表皮生长因子受体在肿瘤中的高度表达及其在肿瘤细胞生长、分化中起重要作用的这些特点，使表皮生长因子受体成为具有良好前景的肿瘤治疗靶点。

[0003] 抗表皮生长因子受体的治疗性抗体，最早来自由表皮生长因子受体高表达表皮癌 A431 免疫小鼠而得到的鼠源抗体 M225。M225 与表皮生长因子受体特异结合，阻断其与配体结合，从而抑制表皮生长因子受体酪氨酸蛋白激酶的活性 (Goldstein et al，1995，Clin.Cancer.Res.1：1311-1318)。鼠源抗体作为外源蛋白，反复刺激人体，可引起超敏反应，被称为人抗鼠抗体反应。鼠源抗体在体内可能被抗抗体中和，进而被快速清除，甚至导致严重的过敏反应而致死。另外，鼠源单克隆抗体缺乏人抗体具有的免疫效应功能，如抗体依赖细胞介导的细胞毒 (ADCC) 和补体依赖的细胞毒 (CDC) 作用，因而疗效不佳。因此人鼠嵌合抗体 C225 抗体 (爱必妥，Erbitux 或 Cetuximab，ImClone(现在 Eli Lilly) 公司) 采用人 IgG1 κ 和鼠抗 M225 的可变区，降低了鼠源单抗的免疫原性，保留了与表皮生长因子受体的特异亲和性，可阻断 EGF 和 TGFa 等配体与表皮生长因子受体的结合，抑制其磷酸化和下游信号传导，从而抑制肿瘤细胞的生长，诱导凋亡，减少基质金属蛋白酶和血管上皮生长因子的产生。2004 年美国 FDA 批准爱必妥治疗结直肠癌，2006 批准治疗头颈部癌，目前有更多临

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床试验用于其它肿瘤适应症。与其他化疗药相比，抗体药物特异性强，副作用小，在临床上取得了较好的疗效。然而，人鼠嵌合爱必妥单抗仍有免疫原性，临床报道其免疫原性为5%，容易产生人抗抗体反应而导致免疫复合物介导的抗体清除，和皮肤及粘膜的副作用 (An Z, et al.,2009, Therapeutic Monoclonal Antibody, p313)。

[0004] 帕尼单抗 (panitumumab, Amgen 公司) 是采用转基因小鼠技术制备的全人化单克隆抗体，无鼠源蛋白序列，靶向作用于表皮生长因子受体 (EGFR)，2006 年 9 月被 FDA 批准上市，与氟嘧啶、奥沙利铂和伊立替康合用或在化疗后用于治疗 EGFR 阳性的转移性直结肠癌。2006 年 FDA 批准其单药治疗化疗耐受的转移性结直肠癌 (mCRC)。然而帕尼单抗是 IgG2 亚型抗体，与 IgG1 相比，IgG2 的 CDC 活性及 ADCC 等生物学活性明显减低 ；另外，IgG2 亚型抗体稳定性较差，这可能是临床效果上全人抗体帕尼单抗与嵌合抗体爱必妥相比没有明显优势的主要原因。

[0005] 因此，本领域亟需具有更低免疫原性和更高生物学活性的人源化抗表皮生长因子受体抗体，以进一步提高疗效，降低副作用。

发明内容

[0006] 本发明人在鼠源抗体的基础上，利用抗体人源化技术，令人惊奇地获得了一种具有更低免疫原性和更高生物学活性的人源化抗表皮生长因子受体抗体，本发明基于以上发现而完成。具体包括以下几个方面 ：

[0007] 本发明第一方面涉及抗表皮生长因子受体抗体或其抗原结合部分，其重链 CDR1 的序列选自 SEQ ID NO :1 和 SEQ ID NO :39 所示序列或其衍生序列，CDR3 的序列选自 SEQ ID NO :2、SEQ ID NO :3 和 SEQ ID NO :40 所示序列或其衍生序列 ；和 / 或

[0008] 其轻链 CDR3 的序列选自 SEQ ID NO :9、SEQ ID NO :10 和 SEQ ID NO :41 所示序列或其衍生序列。

[0009] 根据本发明第一方面的抗体或其抗原结合部分，其重链 CDR2 和轻链 CDR1、CDR2 的序列可以为鼠源或人源或人鼠嵌合的抗表皮生长因子受体抗体的相应部分。

[0010] 根据本发明第一方面任一项的抗体或其抗原结合部分，其重链和轻链 FR 的序列可以为鼠源或人源或人鼠嵌合的抗表皮生长因子受体抗体的相应部分。

[0011] 在本发明的实施方案中，所述抗体或其抗原结合部分的重链可变区 CDR 的序列选自以下 (1) ～ (4) 所示序列或其衍生序列 ：

[0012] (1) CDR1 的序列为 SEQ ID NO :1 所示序列，CDR2 的序列为 SEQ ID NO :4 所示序列，CDR3 的序列为 SEQ ID NO :2 所示序列 ；

[0013] (2) CDR1 的序列为 SEQ ID NO :1 所示序列，CDR2 的序列为 SEQ ID NO :4 所示序列，CDR3 的序列为 SEQ ID NO :40 所示序列 ；

[0014] (3) CDR1 的序列为 SEQ ID NO :39 所示序列，CDR2 的序列为 SEQ ID NO :4 所示序列，CDR3 的序列为 SEQ ID NO :3 所示序列 ；

[0015] (4) CDR1 的序列为 SEQ ID NO :39 所示序列，CDR2 的序列为 SEQ ID NO :4 所示序列，CDR3 的序列为 SEQ ID NO :40 所示序列 ；和 / 或

[0016] 其轻链可变区 CDR 的序列选自以下 (5) ～ (8) 所示序列或其衍生序列 ：

[0017] (5) CDR1 的序列为 SEQ ID NO :11 所示序列，CDR2 的序列为 SEQ ID NO :12 所示序

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列，CDR3 的序列为 SEQ ID NO :9 所示序列；

[0018] (6)CDR1 的序列为 SEQ ID NO :11 所示序列，CDR2 的序列为 SEQ ID NO :12 所示序列，CDR3 的序列为 SEQ ID NO :41 所示序列；

[0019] (7)CDR1 的序列为 SEQ ID NO :11 所示序列，CDR2 的序列为 SEQ ID NO :12 所示序列，CDR3 的序列为 SEQ ID NO :41 所示序列；

[0020] (8)CDR1 的序列为 SEQ ID NO :11 所示序列，CDR2 的序列为 SEQ ID NO :12 所示序列，CDR3 的序列为 SEQ ID NO :10 所示序列。

[0021] 根据本发明第一方面任一项的抗体或其抗原结合部分，其重链 FR1 的序列选自 SEQ ID NO :19 ～ SEQ ID NO :22 所示序列或其衍生序列，FR2 的序列选自 SEQ ID NO :23 ～ SEQ ID NO :25 所示序列或其衍生序列，FR3 的序列选自 SEQ ID NO :26 ～ SEQ ID NO :29 所示序列或其衍生序列，FR4 的序列为 SEQ ID NO :30 所示序列或其衍生序列；和 / 或

[0022] 其轻链 FR1 的序列选自 SEQ ID NO :31 ～ SEQ ID NO :32 所示序列或其衍生序列，FR2 的序列选自 SEQ ID NO :33 ～ SEQ ID NO :34 所示序列或其衍生序列，FR3 的序列选自 SEQ ID NO :35 ～ SEQ ID NO :36 所示序列或其衍生序列，FR4 的序列选自 SEQ ID NO :37 ～ SEQ ID NO :38 所示序列或其衍生序列。

[0023] 在本发明的实施方案中，所述抗体或其抗原结合部分的重链可变区 FR 的序列选自以下 (9) ～ (12) 所示序列或其衍生序列：

[0024] (9)FR1 的序列为 SEQ ID NO :19 所示序列，FR2 的序列为 SEQ ID NO :23 所示的序列，FR3 的序列为 SEQ ID NO :26 所示的序列，FR4 的序列为 SEQ ID NO :30 所示的序列；

[0025] (10)FR1 的序列为 SEQ ID NO :20 所示序列，FR2 的序列为 SEQ ID NO :24 所示的序列，FR3 的序列为 SEQ ID NO :27 所示的序列，FR4 的序列为 SEQ ID NO :30 所示的序列；

[0026] (11)FR1 的序列为 SEQ ID NO :21 所示序列，FR2 的序列为 SEQ ID NO :23 所示的序列，FR3 的序列为 SEQ ID NO :28 所示的序列，FR4 的序列为 SEQ ID NO :30 所示的序列；

[0027] (12)FR1 的序列为 SEQ ID NO :22 所示序列，FR2 的序列为 SEQ ID NO :25 所示的序列，FR3 的序列为 SEQ ID NO :29 所示的序列，FR4 的序列为 SEQ ID NO :30 所示的序列；和 / 或

[0028] 其轻链可变区 FR 的序列选自以下 (13) ～ (16) 所示序列或其衍生序列：

[0029] (13)FR1 的序列为 SEQ ID NO :31 所示序列，FR2 的序列为 SEQ ID NO :33 所示的序列，FR3 的序列为 SEQ ID NO :35 所示的序列，FR4 的序列为 SEQ ID NO :37 所示的序列；

[0030] (14)FR1 的序列为 SEQ ID NO :31 所示序列，FR2 的序列为 SEQ ID NO :33 所示的序列，FR3 的序列为 SEQ ID NO :35 所示的序列，FR4 的序列为 SEQ ID NO :38 所示的序列；

[0031] (15)FR1 的序列为 SEQ ID NO :32 所示序列，FR2 的序列为 SEQ ID NO :34 所示的序列，FR3 的序列为 SEQ ID NO :36 所示的序列，FR4 的序列为 SEQ ID NO :38 所示的序列；

[0032] (16)FR1 的序列为 SEQ ID NO :31 所示序列，FR2 的序列为 SEQ ID NO :33 所示的序列，FR3 的序列为 SEQ ID NO :35 所示的序列，FR4 的序列为 SEQ ID NO :37 所示的序列。

[0033] 在本发明的具体实施方案中，所述抗体或其抗原结合部分的重链可变区序列为 SEQ ID NO :5 所示的序列，轻链可变区序列为 SEQ ID NO :13 所示的序列。

[0034] 在本发明的另一个具体实施方案中，所述抗体或其抗原结合部分的重链可变区序列为 SEQ ID NO :6 所示的序列，轻链可变区序列为 SEQ ID NO :14 所示的序列。

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[0035] 在本发明的另一个具体实施方案中，所述抗体或其抗原结合部分的重链可变区序列为 SEQ ID NO:7 所示的序列，轻链可变区序列为 SEQ ID NO:15 所示的序列。

[0036] 在本发明的另一个具体实施方案中，所述抗体或其抗原结合部分的重链可变区序列为 SEQ ID NO:8 所示的序列，轻链可变区序列为 SEQ ID NO:16 所示的序列。

[0037] 根据本发明第一方面任一项的抗体或其抗原结合部分，其还包含人 IgG1 重链恒定区和人 κ 轻链恒定区。

[0038] 在本发明中，所述 IgG1 重链恒定区包括各种同种异型，如 G1m(f)、G1m(z)、G1m(z,a) 或 G1m(z,a,x)。在本发明的实施方案中，所述 IgG1 重链恒定区为 G1m(z) 型。

[0039] 在本发明中，所述 κ 轻链恒定区包括各种同种异型，如 Km1、Km1,2 或 Km3，在本发明的的实施方案中，所述 κ 轻链恒定区为 Km3 型。

[0040] 根据本发明第一方面任一项的抗体或其抗原结合部分，其中所述抗原结合部分包括单链抗体 (scFv)、嵌合抗体、双抗体、scFv-Fc 二价分子、dAb 和互补决定区 (CDR) 片段、Fab 片段、Fd 片段、Fab′ 片段、Fv 和 $F(ab')_2$ 片段。

[0041] 本发明第二方面涉及分离的核酸分子，其编码本发明第一方面任一项所述的抗体或其抗原结合部分。

[0042] 本发明第三方面涉及载体，其含有本发明第二方面任一项的核酸分子。

[0043] 在本发明中，所述载体可以为克隆载体或表达载体。所述表达载体例如为原核表达载体，真核表达载体，噬菌体载体或病毒载体，均为本领域常用的载体。其中所述原核表达载体例如为 PET28a、pGEM Ex1、pGEM7ZF(+) 或 pBAD24 等，所述真核表达载体例如为 pBudCE4.1、pcDNA3.1、pCMV-Tag2B 或 pGL3 basic 等，在本发明的实施方案中，所述真核表达载体为 pcDNA3.1，所述病毒载体例如为逆转录病毒、慢病毒、腺病毒和腺相关病毒。在本发明的实施方案中，所述病毒载体为腺病毒载体。

[0044] 本发明第四方面涉及宿主细胞，其含有本发明第二方面的核酸分子或第三方面的载体。

[0045] 在本发明中，所述宿主细胞可以为原核细胞或真核细胞。所述真核细胞例如为哺乳动物细胞。所述宿主细胞可以通过向原核细胞或真核细胞中引入 / 转染上述的核酸分子或载体而得到。

[0046] 在本发明中，所述原核细胞例如可以为大肠杆菌，所述真核细胞例如可以为酵母细胞、昆虫细胞 (如 Sf21)、哺乳动物细胞，所述哺乳动物例如可以为 NSO 细胞、CHO 细胞。在本发明的一个实施方案中，所述宿主细胞为真核细胞。在本发明的一个实施方案中，所述真核细胞为 CHO-S 细胞。

[0047] 在本发明中，可以利用本领域所知的任何种类的转染方法获得转染有特定核酸或载体的宿主细胞。例如，可通过电穿孔或显微注射将核酸引入细胞中。或者，可使用脂转染试剂如 FuGENE6、X-tremeGENE 和 LipofectAmine。或者，可通过基于逆转录病毒、慢病毒、腺病毒和腺相关病毒的适当病毒载体将核酸引入细胞中。

[0048] 本发明第五方面涉及组合物，其含有本发明第一方面任一项的抗体或其抗原结合部分，以及任选的一种或多种药学可接受的辅料；优选地，所述辅料选自蛋白水解酶抑制剂、药用载体、稀释剂和佐剂。

[0049] 本发明第六方面涉及本发明第一方面任一项的抗体或其抗原结合部分、或第二方

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面的核酸分子、或第三方面的载体、或第四方面的宿主细胞在制备预防 / 治疗表皮生长因子或表皮生长因子受体相关疾病的药物中的用途。

[0050] 在本发明中，所述表皮生长因子或表皮生长因子受体相关疾病是指由于表皮生长因子高表达或表皮生长因子受体高表达而引起的疾病。在本发明的实施方案中，所述表皮生长因子或表皮生长因子受体相关疾病是指恶性肿瘤，其主要是指发生于上皮组织的恶性肿瘤，其包括但不限于直结肠癌、肝癌、头颈部癌、肺癌、卵巢癌、宫颈癌、阴道癌、膀胱癌、食管癌、口腔癌、皮肤癌、乳腺癌、前列腺癌和胰腺癌等。

[0051] 本发明第七方面涉及检测试剂或试剂盒，其含有本发明第一方面任一项的抗体或其抗原结合部分；

[0052] 优选地，所述抗体或其抗原结合部分还可包含可检测的标记；或者

[0053] 优选地，所述检测试剂或试剂盒还可包含第二抗体，其特异性识别所述抗体或其抗原结合部分；或者

[0054] 优选地，所述第二抗体还可包含可检测的标记。

[0055] 本发明第八方面涉及本发明第一方面任一项的抗体或其抗原结合部分在制备检测试剂或试剂盒中的用途，其中所述检测试剂或试剂盒用于检测表皮生长因子或表皮生长因子受体，或者用于诊断表皮生长因子或表皮生长因子受体相关疾病。

[0056] 以下对本发明做进一步描述。

[0057] 本发明的目的是针对已有的抗表皮生长因子受体抗体，利用去免疫原性的抗体人源化技术改变抗体的 CDR 区和 / 或框架区，以获得具有更低免疫原性和更高生物学活性的抗表皮生长因子受体抗体。

[0058] 在本发明的一个实施方案中，首先分析鼠抗 M225 的重链可变区的序列，根据其重链可变区 FR1，FR2，FR3 和 FR4 序列对人抗体基因序列库进行比较，找出与鼠抗 M225 重链可变区 FR1，FR2，FR3 和 FR4 序列相似的人胚系 (germline) 抗体 (比成熟抗体免疫原性更低) 可变区相对应序列。然后经过 insilicon 方法，分析相似序列与 HLA-DR 分子结合的亲和性，选出亲和性最低的框架序列，从而最终确立重链可变区的 FR1，FR2，FR3 和 FR4 的人源化序列。在此框架结构的基础上，应用计算机分子模型分析鼠 M225 抗体的可变区立体空间结构，分析出支持 CDR 构型所需保留的鼠 M225 抗体对应框架氨基酸残基。对 HLA-DR 分子结合的亲和性较高的 CDR1 和 CDR3 的去免疫原化，则应用分子对接，分析表皮生长因子受体抗原鼠 M225 抗体靶位及其周边氨基酸残基与人源化重链可变区的立体空间结合方式，通过计算静电力，溶液媒合 (salvation)，范德华引力和熵值，选择 CDR1 和 CDR3 序列中可以优化的氨基酸，以降低于 HLA-DR 分子结合的亲和性，并改善抗体 CDR3 区域与表皮生长因子受体抗原表位的结合。

[0059] 在本发明的另一个实施方案中，分析鼠抗 M225 抗体的轻链可变区的序列，根据其轻链可变区 FR1，FR2，FR3 和 FR4 序列对人抗体基因序列库 (NCBI Ig BLAST) 进行比较，找出与鼠抗 M225 轻链可变区 FR1，FR2，FR3 和 FR4 序列相似的人胚系 (germline) 抗体可变区相对应序列，然后经过 in silicon 分析相似序列与 HLA-DR 分子结合的亲和性，选出亲和性最低的框架序列，从而最终确立轻链可变区的 FR1，FR2，FR3 和 FR4 的人源化序列。在此框架结构的基础上，应用计算机分子模型分析鼠 225 抗体的可变区立体空间结构，分析出支持 CDR 构型所需保留的鼠 M225 抗体对应框架氨基酸残基。对 HLA-DR 分子结合的亲和性较

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高的 CDR1 和 CDR3 的去免疫原化，则应用分子对接，分析表皮生长因子受体抗原鼠 M225 抗体靶位及其周边氨基酸残基与人源化重链可变区的立体空间结合方式，通过计算静电力，溶液媒合 (salvation)，范德华引力和熵值，选择 CDR1 和 CDR3 序列中可以优化的氨基酸，以降低于 HLA-DR 分子结合的亲和性，并改善抗体 CDR3 区域与表皮生长因子受体抗原表位的结合。

[0060] 在本发明的实施方案中，从正常人 B 细胞中分别获得人 IgG1 重链恒定区 Fc 片段和轻链恒定区 κ 片段，并分别和上述重链可变区和轻链可变区连接，构建抗体重链和轻链表达载体。将所述表达载体共转染哺乳动物细胞进行表达、纯化，得到人源化抗表皮生长因子受体抗体 BA03、BB03、BC03 和 BD03。

[0061] 在本发明的实施方案中，所述抗体在与细胞表面表皮生长因子受体的亲和性、阻断 EGF 配体与细胞表面表皮生长因子受体结合的活性、抑制细胞表面表皮生长因子受体磷酸化的活性和 ADCC 作用方面，与爱必妥相比均具有更高的活性，同时具有更低的免疫原性。

[0062] 在本发明的实施方案中，所述抗体对多种肿瘤细胞的生长都具有抑制作用，例如表皮癌、肺癌、结肠癌、胰腺癌和乳腺癌，并且在动物水平具有明显的抑制肿瘤生长的作用。

[0063] 在本发明中，除非另有说明，否则本文中使用的科学和技术名词具有本领域技术人员所通常理解的含义。并且，本文中所用的蛋白质和核酸化学、分子生物学、细胞和组织培养、微生物学、免疫学相关术语和实验室操作步骤均为相应领域内广泛使用的术语和常规步骤。同时，为了更好地理解本发明，下面提供相关术语的定义和解释。

[0064] 在本发明中，术语“抗体”是指通常由两对相同的多肽链（每对具有一条“轻”(L) 链和一条“重”(H) 链）组成的免疫球蛋白分子。抗体轻链可分类为 κ 和 λ 轻链。重链可分类为 μ、δ、γ、α 或 ε，并且分别将抗体的同种型定义为 IgM、IgD、IgG、IgA 和 IgE。在轻链和重链内，可变区和恒定区通过大约 12 或更多个氨基酸的“J”区连接，重链还包含大约 3 个或更多个氨基酸的“D”区。各重链由重链可变区 (V_H) 和重链恒定区 (C_H) 组成。重链恒定区由 3 个结构域 (C_H1、C_H2 和 C_H3) 组成。各轻链由轻链可变区 (V_L) 和轻链恒定区 (C_L) 组成。轻链恒定区由一个结构域 C_L 组成。抗体的恒定区可介导免疫球蛋白与宿主组织或因子，包括免疫系统的各种细胞（例如，效应细胞）和经典补体系统的第一组分 (C1q) 的结合。V_H 和 V_L 区还可被细分为具有高变性的区域（称为互补决定区 (CDR)），其间散布有较保守的称为构架区 (FR) 的区域。各 V_H 和 V_L 由按下列顺序：FR1、CDR1、FR2、CDR2、FR3、CDR3、FR4 从氨基末端至羧基末端排列的 3 个 CDR 和 4 个 FR 组成。各重链 / 轻链对的可变区 (V_H 和 V_L) 分别形成抗体结合部位。氨基酸至各区域或结构域的分配遵循 Kabat Sequences of Proteins of Immunological Interest(National Institutes of Health, Bethesda, Md.(1987 and 1991))，或 Chothia& Lesk(1987)J.Mol.Biol.196:901-917；Chothia 等人 (1989)Nature342:878-883 的定义。术语“抗体”不受任何特定的产生抗体的方法限制。例如，其包括，特别地，重组抗体、单克隆抗体和多克隆抗体。抗体可以是不同同种型的抗体，例如，IgG（例如，IgG1，IgG2，IgG3 或 IgG4 亚型），IgA1，IgA2，IgD，IgE 或 IgM 抗体。

[0065] 在本发明中，术语抗体的“抗原结合部分”是指全长抗体的一个或多个部分，所述部分保持结合抗体所结合的相同抗原（例如，ERCC1）的能力，与完整抗体竞争对抗原的特异性结合。通常参见，Fundamental Immunology, Ch.7(Paul, W., ed., 第 2 版，Raven Press,

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N.Y.(1989),其以其全文通过引用合并入本文,用于所有目的。可通过重组DNA技术或通过完整抗体的酶促或化学断裂产生抗原结合部分。在一些情况下,抗原结合部分包括Fab、Fab′、$F(ab')_2$、Fd、Fv、dAb和互补决定区(CDR)片段、单链抗体(例如,scFv)、嵌合抗体、双抗体(diabody)和这样的多肽,其包含足以赋予多肽特异性抗原结合能力的抗体的至少一部分。

[0066] 在本发明中,术语"Fd片段"意指由V_H和C_H1结构域组成的抗体片段;术语"Fv片段"意指由抗体的单臂的V_L和V_H结构域组成的抗体片段;术语"dAb片段"意指由V_H结构域组成的抗体片段(Ward等人,Nature341:544-546(1989));术语"Fab片段"意指由V_L、V_H、C_L和C_H1结构域组成的抗体片段;术语"$F(ab')_2$片段"意指包含通过铰链区上的二硫桥连接的两个Fab片段的抗体片段。

[0067] 在一些情况下,抗体的抗原结合部分是单链抗体(例如,scFv),其中V_L和V_H结构域通过使其能够产生为单个多肽链的连接体配对形成单价分子(参见,例如,Bird等人,Science 242:423-426(1988)和Huston等人,Proc.Natl.Acad.Sci.USA 85:5879-5883(1988))。此类scFv分子可具有一般结构:NH_2-V_L-接头-V_H-COOH或NH_2-V_H-接头-V_L-COOH。合适的现有技术接头由重复的GGGGS氨基酸序列或其变体组成。例如,可使用具有氨基酸序列$(GGGGS)_4$的接头,但也可使用其变体(Holliger等人(1993),Proc.Natl.Acad.Sci.USA 90:6444-6448)。可用于本发明的其他接头由Alfthan等人(1995),Protein Eng.8:725-731,Choi等人(2001),Eur.J.Immunol.31:94-106,Hu等人(1996),Cancer Res.56:3055-3061,Kipriyanov等人(1999),J.Mol.Biol.293:41-56和Roovers等人(2001),Cancer Immunol.描述。

[0068] 在一些情况下,抗体是双抗体,即,双价抗体,其中V_H和V_L结构域在单个多肽链上表达,但使用太短的连接体以致不允许在相同链的两个结构域之间配对,从而迫使结构域与另一条链的互补结构域配对并且产生两个抗原结合部位(参见,例如,Holliger P.等人,Proc.Natl.Acad.Sci.USA90:6444-6448(1993),和Poljak R.J.等人,Structure 2:1121-1123(1994))。

[0069] 在本发明中,术语"衍生序列"是指与本发明的抗体或抗原结合部分相应片段的序列高度同源且仍保留与本发明的抗体或抗原结合部分相应片段具有相似的EGFR结合活性和免疫原性的序列。

[0070] 可使用本领域技术人员已知的常规技术(例如,重组DNA技术或酶促或化学断裂法)从给定的抗体(例如单克隆抗体2E12)获得抗体的抗原结合部分(例如,上述抗体片段),并且以与对于完整抗体的方式相同的方式就特异性筛选抗体的抗原结合部分。

[0071] 在本发明中,20种常规氨基酸和其缩写遵从常规用法。参见Immunology-A Synthesis(第2版,E.S.Golub和D.R.Gren,Eds.,Sinauer Associates,Sunderland,Mass.(1991)),其通过引用合并入本文。

[0072] 在本发明中,所述核酸包括DNA和RNA。

[0073] 发明的有益效果

[0074] 本发明针对已经上市的人鼠嵌合爱必妥单抗,利用去免疫原性的抗体人源化技术改变抗体的框架区和部分引起免疫原性的CDR区,形成全新序列的人源化抗表皮生长因子受体IgG1亚型抗体分子,所得抗体具有比爱必妥单抗更低的免疫原性和更高的的靶点结

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合活性，从而有更强的生物学活性和体内外抗肿瘤活性，可以预期临床应用副作用更低，疗效更好。

附图说明

[0075] 图 1 所示为抗体与细胞表面表皮生长因子受体亲和力的比较。

[0076] 图 2 所示为抗体阻断 EGF 配体与细胞表面表皮生长因子受体结合的比较，其中纵坐标为 OD450 值。

[0077] 图 3 所示为抗体抑制 A431 细胞表面表皮生长因子受体磷酸化的比较。

[0078] 图 4 所示为抗体依赖性细胞介导的细胞毒 (ADCC) 活性测定。

[0079] 图 5 所示为树突状细胞 -T 细胞激活实验测定抗体体外免疫原性。

[0080] 图 6 所示为抗体对人表皮癌 A431 小鼠肿瘤模型的生长抑制作用。

具体实施方式

[0081] 下面将结合实施例对本发明的实施方案进行详细描述，但是本领域技术人员将会理解，下列实施例仅用于说明本发明，而不应视为限定本发明的范围。实施例中未注明具体条件者，按照常规条件或制造商建议的条件进行。所用试剂或仪器未注明生产厂商者，均为可以通过市购获得的常规产品。

[0082] 实施例 1、抗表面表皮生长因子受体人源化单克隆抗体重链可变区序列的设计

[0083] 以下为鼠抗 M225 的重链可变区的序列，下划线部分为 FR1，FR2，FR3 和 FR4 框架序列：QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYEFAYWGQGTLVTVSA(SEQ ID NO：17)。

[0084] 根据其重链可变区 FR1，FR2，FR3 和 FR4 序列对人抗体基因序列库进行比较，找出与鼠抗 M225 重链可变区 FR1，FR2，FR3 和 FR4 序列相似的人胚系 (germline) 抗体可变区相对应序列。然后用 NetMHCIIpan 软件或自编软件，经过 in silicon 方法，分析相似序列与 HLA-DR 分子结合的亲和性，选出亲和性最低的框架序列，从而最终确立人源化重链可变区的 FR1，FR2，FR3 和 FR4 的序列。在此框架结构的基础上，应用计算机分子模型分析鼠 225 抗体的可变区立体空间结构，分析出支持 CDR 构型所需保留的鼠 225 抗体对应框架氨基酸残基 L29，L48 和 L67。对 HLA-DR 分子结合的亲和性较高的 CDR1 和 CDR3 的去免疫原化，则应用分子对接，分析表皮生长因子受体鼠源 M225 抗体靶位及其周边氨基酸残基与人源化重链可变区的立体空间结合方式，通过计算静电力，溶液媒合 (salvation)，范德华引力和熵值 (Sircar et al，SnugDock：paratope structural optimization during antibody-antigen docking compensates for errors in antibody homology models，PLoS Comput Biol. 2010 Jan 22；6)，选择 CDR1 和 CDR3 序列中可以优化的氨基酸，将 CDR1 中的 33 位点 GLy 优化为氨基酸 Asp，将 CDR3 中的 100 位点 Thr 优化为氨基酸 Asp，以降低与 HLA-DR 分子结合的亲和性，并改善抗体 CDR3 区域与表皮生长因子受体抗原表位的结合。新设计的重链 CDR1 为：N Y D V H(SEQ ID NO：1)；新设计的重链 CDR3 序列为 A L D Y Y D Y E F A Y(SEQ ID NO：2) 和 A L T Y Y D Y D F A Y(SEQ ID NO：3)，由于 CDR2 对 HLA-DR 分子结合的亲和性较低，保留原鼠抗 M225 的 CDR2 序列 (其序列为 VIWSGGNTDYNTPFTS，SEQ ID NO：4)。因此，整合优化的框架序列和 CDR 序列，得到的 4 个新型抗表皮生长因子受体人

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源化单克隆抗体重链可变区序列为：

[0085] QVQLQESGPGLVKPSETLSLTCTVSGFSLSNYDVHWVRQAPGKGLEWLGVIWSGGNTDYNTPFTSRLTISVDTSKNQFSLKLSSVTAADTAVYYCARALDYYDYEFAYWGQGTLVTVSS(SEQ ID NO:5)；

[0086] QVQLQESGPGLVKPSETLSLTCTVSGFSLTNYDVHWIRQPPGKGLEWIGVIWSGGNTDYNTPFTSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARALTYYDYEFAYWGQGTLVTVSS(SEQ ID NO:6)；

[0087] EVQLVESGGGLVQPGGSLRLSCAASGFSLTNYGVHWVRQAPGKGLEWLGVIWSGGNTDYNTPFTSRLTISKDNSKNTLYLQMNSLRAEDTAVYYCARALTYYDYDFAYWGQGTLVTVSS(SEQ ID NO:7)；

[0088] QVQLKESGPGLVAPSQSLSITCTVSGFSLTNYGVHWVRQPPGKGLEWLGVIWSGGNTDYNTPFTSRLSISKDNSKSQVFLRMYSLQTDDTARYYCARALTYYDYEFAYWGQGTLVTVSS(SEQ ID NO:8)。

[0089] 以上各序列中的各小段序列编号如下：

[0090] QVQLQESGPGLVKPSETLSLTCTVSGFSLS(SEQ ID NO:19)

[0091] QVQLQESGPGLVKPSETLSLTCTVSGFSLT(SEQ ID NO:20)

[0092] EVQLVESGGGLVQPGGSLRLSCAASGFSLT(SEQ ID NO:21)

[0093] QVQLKESGPGLVAPSQSLSITCTVSGFSLT(SEQ ID NO:22)

[0094] WVRQAPGKGLEWLG(SEQ ID NO:23)

[0095] WIRQPPGKGLEWIG(SEQ ID NO:24)

[0096] WVRQPPGKGLEWLG(SEQ ID NO:25)

[0097] RLTISVDTSKNQFSLKLSSVTAADTAVYYCAR(SEQ ID NO:26)

[0098] RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR(SEQ ID NO:27)

[0099] RLTISKDNSKNTLYLQMNSLRAEDTAVYYCAR(SEQ ID NO:28)

[0100] RLSISKDNSKSQVFLRMYSLQTDDTARYYCAR(SEQ ID NO:29)

[0101] WGQGTLVTVSS(SEQ ID NO:30)

[0102] NYGVH(SEQ ID NO:39)

[0103] ALTYYDYEFAY(SEQ ID NO:40)。

[0104] 实施例 2、抗表皮生长因子受体人源化单克隆抗体轻链可变区序列的设计

[0105] 以下为鼠抗 M225 的轻链可变区的序列，下划线部分为 FR1，FR2，FR3 和 FR4 框架序列：

[0106] DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELK(SEQ ID NO:18)。

[0107] 同样分析鼠抗 225 抗体的轻链可变区的序列，根据其轻链可变区 FR1，FR2，FR3 和 FR4 序列对人抗体基因序列库进行比较，找出与鼠抗 M225 轻链可变区 FR1，FR2，FR3 和 FR4 序列相似的人胚系 (germline) 抗体可变区相对应序列，然后用 NetMHCIIpan 软件或自编软件，经过 in silicon 分析所选相似序列与 HLA-DR 分子结合的亲和性，选出亲和性最低的框架序列，从而最终确立轻链可变区的 FR1，FR2，FR3 和 FR4 的人源化序列。在此框架结构的基础上，应用计算机分子模型分析鼠 225 抗体的可变区立体空间结构，分析出支持 CDR 构型所需保留的鼠抗体对应框架氨基酸残基 I58。对 HLA-DR 分子结合的亲和性较高的 CDR3 的去免疫原化，则应用分子对接，分析表皮生长因子受体鼠源 M225 抗体靶位及其周边氨基酸残基与人源化重链可变区的立体空间结合方式，通过计算静电力，溶液媒合 (salvation)，范德华引力和熵值 (Sircar et al，SnugDock：paratope structural optimization during

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antibody-antigen docking compensates for errors in antibody homology models, PLoS Comput Biol. 2010 Jan 22 ;6)，选择 CDR3 序列中可以优化的氨基酸，将 CDR3 中的 93 位点的 Asn 优化为氨基酸 Glu，97 位点的 Thr 优化为氨基酸 Ser，以降低于 HLA-DR 分子结合的亲和性，并改善抗体 CDR3 区域与表皮生长因子受体抗原表位相邻带正电荷氨基酸的结合。新设计的轻链 CDR3 为 :Q Q N N E W PT S(SEQ ID NO :9) 和 Q Q N N D W P T T(SEQ ID NO :10)。由于轻链 CDR1 和 CDR2 对 HLA-DR 分子结合的亲和性较低，保留原鼠抗 M225 的 CDR1 (其序列为 RASQSIGTNIH, SEQ ID NO :11) 和 CDR2 序列 (其序列为 YASESIS, SEQ ID NO :12)。因此，整合优化的框架序列和 CDR 序列，得到的新型抗表面表皮生长因子受体人源化单克隆抗体轻链可变区序列为 :

[0108] EIVLTQSPDFQSVTPKEKVTITCRASQSIGTNIHWYQQKPDQSPKLLIKYASESISGIPSRFSGSGSGT DFTLTINSLEAEDAATYYCQQNNEWPTSFGQGTKLEIK(SEQ ID NO :13) ;

[0109] EIVLTQSPDFQSVTPKEKVTITCRASQSIGTNIHWYQQKPDQSPKLLIKYASESISGIPSRFSGSGSGT DFTLTINSLEAEDAATYYCQQNNNWPTTFGAGTKLEIK(SEQ ID NO :14)

[0110] DIVLTQSPATLSVTPGESVSFSCRASQSIGTNIHWYQQKSHESPRLLIKYASESISGIPSRFSGSGSGT DFTLSINSVETEDFGMYYCQQNNNWPTTFGAGTKLEIK(SEQ ID NO :15)

[0111] EIVLTQSPDFQSVTPKEKVTITCRASQSIGTNIHWYQQKPDQSPKLLIKYASESISGIPSRFSGSGSGT DFTLTINSLEAEDAATYYCQQNNDWPTTFGQGTKLEIK(SEQ ID NO :16)。

[0112] 以上各序列中的各小段序列编号如下 :

[0113] EIVLTQSPDFQSVTPKEKVTITC(SEQ ID NO :31)

[0114] DIVLTQSPATLSVTPGESVSFSC(SEQ ID NO :32)

[0115] WYQQKPDQSPKLLIK(SEQ ID NO :33)

[0116] WYQQKSHESPRLLIK(SEQ ID NO :34)

[0117] GIPSRFSGSGSGTDFTLTINSLEAEDAATYYC(SEQ ID NO :35)

[0118] GIPSRFSGSGSGTDFTLSINSVETEDFGMYYC(SEQ ID NO :36)

[0119] FGQGTKLEIK(SEQ ID NO :37)

[0120] FGAGTKLEIK(SEQ ID NO :38)

[0121] QQNNNWPTT(SEQ ID NO :41)。

[0122] 根据上述实施例得到的人源化抗体有以下组合 :

[0123] 表 1. 人源化抗 EGFR 抗体组合

[0124]

抗体名称	抗体重链可变区	抗体轻链可变区
BA03	SEQ ID NO: 5	SEQ ID NO: 13
BB03	SEQ ID NO: 6	SEQ ID NO: 14
BC03	SEQ ID NO: 7	SEQ ID NO: 15
BD03	SEQ ID NO: 8	SEQ ID NO: 16

[0125] 实施例 3、抗表皮生长因子受体人源化单克隆抗体的制备

[0126] (1) 根据实施例 1 和 2 确定的抗体重链可变区序列和轻链可变区序列，分别设计并

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合成编码重链和轻链可变区序列的 PCR 引物寡核苷酸片段，合成时引入需要的酶切位点。PCR 引物寡核苷酸片段一般长约 54 个碱基，相邻寡核苷酸片段约有 18 个碱基重叠。混合同等量的各个 PCR 引物片段，进行重叠延伸 PCR 反应。

[0127] PCR 反应体系：dNTPs 0.2μM（最终浓度）；每个 PCR 引物片段 1μl；10×buffer 3μl；cloned pfu(Invitrogen)1μl；加水至 30μl。

[0128] PCR 反应条件：94℃ 3min → (94℃ 30s → 56℃ 30s → 72℃ 1min)×30 → 72℃ 10min

[0129] PCR 产物经 1% 琼脂糖凝胶电泳分离后回收纯化目的片段，经 EcoRI 酶切后，将抗体重链可变区序列和轻链可变区序列分别克隆到 pCR-BluntII-TOPO(Invitrogen) 载体中，转化到大肠杆菌 TOP10(Invitrogen) 后在 LB/Kanamycin 平板上进行筛选，取 10 个白色菌斑接种于含有 Kanamycin 的 LB 液体培养液中培养，用 QIAGEN 质粒提取试剂盒 (QIAquick PCR purification kit) 提取质粒并进行测序，确定重链和轻链可变区序列。

[0130] (2) 构建表达载体

[0131] 从正常人 B 细胞分离 RNA，用 RT-PCR 方法获得人 IgG1 重链恒定区 IgG1Fc 片段和轻链恒定区 κ 片段，在 RT-PCR 时引入需要的酶切位点，并将其预先构建到 pcDNA3.1(Invitrogen) 表达载体中，分别得到 pcDNA3.1-Fc 和 pcDNA3.1-κ 表达载体，经 DH5 细菌转化，质粒提取和测序确定阳性克隆。

[0132] 其中人 IgG1 重链恒定区 IgG1Fc 片段的序列为：

[0133] ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVITVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK(SEQ ID NO:42)

[0134] 其中轻链恒定区 κ 片段的序列为：

[0135] RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC(SEQ ID NO:43)

[0136] 抗体的重链可变区由 Eco47III/NheI 从 pCR-BluntII-TOPO 的阳性克隆切下，克隆连入 pcDNA3.1-Fc 表达载体中，使重链可变区与恒定区连接。抗体的轻链可变区由 AscI/BsiWI 从 pCR-BluntII-TOPO 的阳性克隆切下，克隆连入 pcDNA3.1-κ 表达载体中，使轻链可变区与恒定区连接（可参考文献 Morrison SL. Cloning, expression, and modification of antibody V regions. Curr Protoc Immunol. 2002 May; Chapter 2: Unit 2.12.）。再次经过 DH5 细菌转化，质粒提取和测序确定阳性克隆。测序结果与记录的抗体的编码序列一致，即分别得到抗体重链和轻链的表达载体。

[0137] (3) 转染 CHO 细胞及特异抗体表达

[0138] 细胞系及培养条件：CHO-S 细胞（购自 Invitrogen）培养条件为 1×CD-CHO（购自 GIBCO），1×HT（购自 GIBCO），8mM 谷氨酰胺（购自 GIBCO），培养于 37℃，8% CO_2 温箱。将步骤 (2) 获得的抗体重链表达载体和轻链表达载体共转染 CHO-S 细胞，采用 DMRIE-C 转染试剂盒（购自 Invitrogen），按照试剂盒说明书操作。转染后的第三天，细胞培养于上述的培养液并加有 500μg/ml G418（购自 GIBCO）和 12.5μg/ml 嘌呤霉素（购自 Sigma）进行加压

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筛选。加压约 14 天后，挑选出的阳性多克隆培养于六孔板中，并用直接 ELISA 的方法（包被 EGFR-Fc 来自表达的 293T 细胞中）检测特异抗体表达量。挑选出表达率最高的阳性多克隆，大批量培养 7 天后，离心收集培养上清，采用蛋白 A 的亲和层析柱（购自 GE）纯化后，透析至 PBS，并经过 0.22um 膜过滤后，即分别得到纯化的抗体 BA03、BB03、BC03 和 BD03，用于以下各实施例。

[0139] 实施例 4 流式细胞仪检测抗体与细胞表面表皮生长因子受体的亲和活性

[0140] 用 A431 细胞 (ATCC) 检测表达抗体对表皮生长因子受体的特异性亲和能力。A431 细胞与不同浓度的产物抗体结合，4℃，1 小时。用 PBS 洗三遍，加入 100 倍稀释的 FITC 标记的抗人 Fcγ 特异性二抗（购自 The Jakson Laboratory），4℃一小时。PBS 洗三遍后用流式细胞仪检测结合的荧光信号（图 1）。根据平均荧光强度利用 Prism 软件计算 EC50（半数有效浓度）值，结果见表 2。其结果表明本发明的人源化抗体 BA03、BD03 比爱必妥（购于百泰生物药业有限公司）有更高结合活性，BB03 和 BC03 与爱必妥相似。所有四个抗体的结合活性均强于泰欣生 (Nimotuzumab，购于 Merck KgaA)。

[0141] 表 2 抗体与 EGFR 结合活性的 EC50 值

[0142]

抗体	EC50(μg/ml)
BA03	0.21
BB03	0.25
BC03	0.32
BD03	0.23
爱必妥	0.28
泰欣生	0.42

[0143] 实施例 5 抗体阻断 EGF 与细胞表面表皮生长因子受体结合

[0144] 用 A431 细胞 (ATCC)Cell-ELISA 方法（请参见 Bioo Scientific 公司 MaxDiscovery in Cell ELISA kit 说明书）检测表达抗体对 EGF 与细胞表面表皮生长因子受体结合的阻断能力。不同浓度的抗体与 A431 细胞在 4℃孵育 1 小时后加入生物素标记的重组人表皮生长因子 EGF（购自 R&D），用 PBS 洗三遍。然后用 HRP 标记的亲和素检测与细胞表面表皮生长因子受体结合的生物素标记的重组人表皮生长因子（图 2）。根据测得的 OD450 值利用 Prism 软件计算 IC50（半数抑制浓度）值，结果见表 3。其结果表明本发明的人源化抗体 BA03、BD03 比爱必妥有更强的阻断配体与细胞表面表皮生长因子受体结合的活性，BB03 和 BC03 与爱必妥相似。四个抗体的阻断活性均强于强于泰欣生。

[0145] 表 3 抗体阻断 EGF 与 EGFR 结合活性的 IC50 值

[0146]

抗体	IC50(μg/ml)

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BA03	2.31
BB03	4.38
BC03	3.59
BD03	2.91
爱必妥	3.25
泰欣生	8.25

[0147] 实施例 6 抗体抑制 A431 细胞表面表皮生长因子受体磷酸化

[0148] 用 6 孔板接种 5×10^5 个 / 孔 A431 细胞，用完全培养基 (DMEM+10％ FBS) 过夜培养。换用 2ml DMEM 培养，使细胞缺乏营养 16-18 小时。再换用 1ml 预热新鲜的培养基 (DMEM，不含 10％ FBS) 培养 1-2 小时。在用 50ng/ml 重组 EGF(表皮生长因子，购自 R&D systems) 刺激前 60 分钟，加 5ug/ml 抗表皮生长因子受体抗体到 A431 细胞。用预冷的 1×PBS 洗 3 遍，加 250μl 细胞裂解液 (cell lysis buffer，购自 Cell Signalling) 裂解后，离心，14000rpm，4℃，15 分钟，收集裂解液，用 BCA 试剂盒蛋白定量。经 western blot 方法，分别用 anti-pEGFR(pY1068) 抗体 (购自 Abnova) 测定表皮生长因子受体磷酸化，用抗总表皮生长因子受体抗体 (购自 Abnova) 测定总表皮生长因子受体含量。其结果表明本发明的人源化抗体 BA03 比爱必妥有更强的抑制 A431 细胞表面表皮生长因子受体磷酸化的活性。两者均强于泰欣生 (图 3)。

[0149] 实施例 7 体外抗体依赖性细胞介导的细胞毒 (ADCC) 试验

[0150] 正常志愿者获取的血液吸入至肝素化的注射器中，与相同体积不含 Ca^{2+}/Mg^{2+} 的 HBSS(Life Technologies) 混合，置于淋巴细胞梯度分离剂 (Life Technologies) 上分层，并且在 800g 离心 30 分钟。收集在分界面上的 PBMC，在 HEPES 缓冲盐水中洗涤，并且再次悬浮于测定培养基，即含 1％热灭活 FBS(HyClone Laboratories，Logan，UT)，2nM L- 谷氨酰胺，10mM HEPES，和 50mg/mL 庆大霉素的 RPMI 1640 培养基 (Life Technologies) 中。将置于 50mL 分析缓冲液中的 Ramos 细胞 (ATCC)(10^4 个细胞 / 孔) 和各种浓度的抗体 (置于 50mL 分析缓冲液中) 加入到圆底的 96 孔培养板上。将此混合物在 37℃进行预培养 30 分钟。接着将 50μL 的 5×10^5 效应细胞分配于各孔并且在 37℃孵育 4h。使用 40 ： 1 的效应细胞 (PBMC) ：靶细胞 (Ramos 细胞) 比率进行实验。该培养板在 250g 离心 10 分钟，然后收集上清液。使用细胞毒性检测试剂盒 (Boehringer Mannheim，Indianapolis，IN) 及制造商的说明来检测上清液中的乳酸脱氢酶活性，根据样本的平均吸光率来计算毒性的百分比。特异性裂解率＝ (平均实验释放 - 平均自发释放)/(平均最大释放 - 平均自发释放). 其结果如图 4 所示，本发明的人源化抗体 BA03、BD03 比爱必妥 (cetuximab) 有更高的 ADCC 活力，在 0.1ug/ml 和 1ug/ml 均高于爱必妥 ($p<0.05$，Student’s t 检验)，BB03 和 BC03 与爱必妥相似。

[0151] 实施例 8 树突状细胞 -T 细胞激活实验测定抗体体外免疫原性

[0152] 将从正常志愿者 ($n=12$) 获取的血液吸入至肝素化的注射器中，与相同

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体积不含 $Ca2^{+}/Mg2^{+}$ 的 HBSS(Life Technologies) 混合，置于淋巴细胞梯度分离剂 (Life Technologies) 上分层，并且在 800g 离心 30 分钟。收集在分界面上的外周血单核细胞，在 HEPES 缓冲盐水中洗涤，并且再次悬浮于测定培养基 RPMI 1640 培养基 (Life Technologies) 中。所得外周血单核细胞继续在 50ml 添加 1 ： 100 稀释的 beta-mercaptoethanol 无血清 AIM V 培养基 (Gibco) 贴壁培养 2 小时收获单核细胞。加入 800units/ml 的 GM-CSF(Endogen) 和 500units/ml 的 IL-4(Endogen) ；继续培养 5 天。然后再加入 0.2units/ml 的 TNF α (Endogen) 和终浓度为 50units/ml 的 IL-1 α (Endogen) 继续培养 2 天。第 7 天，加入 50mg/ml 的 Mitomycin C1 小时，以停止树突状细胞的继续分化，然后以 600G 离心收集分化成熟的树突状细胞。以 2×10^{4} 细胞 / 孔 (100ul) 接种于一个圆底的 96 孔培养板。用 $CD4^{+}$Cellect Kit(Biotex) 的负向选择来收集人 $CD4^{-}$T 细胞在含有 2％人血清的 AIMV 培养基。在含有 100μl 树突状细胞 (1×10^{4} 个 /ml) 的 96 孔培养板中加入 10μl、浓度为 5mM 的检测抗体 (对照组加入 DMSO)，然后每孔加入 100μl 的 $CD4^{+}$T 细胞 (1×10^{5} 个 /ml)。激活的 T 细胞增殖数用 alamarblue 试剂测定 (测定方法参照 Invitrogen 的 alamaraBlue Assay kit 试剂盒说明书)。将结果换算成 T 细胞激活指数 (计算方法为 T 细胞激活指数＝抗体组的 T 细胞增殖数 /DMSO 组的 T 细胞增殖数，可参考文献 Stickler MM et al. A human dendritic cell-based method to identify CD4+T-cell epitopes in potential protein allergens. Environ Health Perspect. 2003 February ;111(2) : 251-254.)。其结果如图 5 所示，本发明的四个人源化抗体比人鼠嵌合抗体爱必妥有更低的免疫原性，其中 BA03 和 BB03 与爱比妥相比有显著降低 ($p < 0.05$, Student's t 检验)。

[0153] 实施例 9 BA03 对肺癌，结肠癌，胰腺癌和乳腺癌肿瘤细胞的体外抗瘤活性

[0154] BA03 对肺癌，结肠癌，胰腺癌和乳腺癌肿瘤细胞的体外抗瘤活性，分别对各肿瘤细胞的 ADCC 活性和体外增长抑制作用进行了探讨。ADCC 活性测定方法与实施例 7 相同。体外增长抑制作用测定方法如下 ：用 96 孔板接种 300-2000 个 / 孔不同肿瘤细胞 (根据不同肿瘤细胞特性确定接种细胞密度)，在完全培养基中加入终浓度为 10μg/ml 的 BA03 和 IgG1 对照抗体 (购自 R&D systems) 培养 5-10 天后，加入 Alamablue 测定细胞活性 (测定方法参照 Invitrogen 的 alamaraBlue Assay kit 试剂盒说明书)。与 IgG1 对比计算相对细胞比活性 (％ ADCC) 和细胞生长抑制率 . 每个肿瘤细胞株生长抑制试验至少重复两次 (三个复孔)。肿瘤细胞中加入抗体后，再加入 PE 荧光标记的二抗 (PE-labled anti-human IgG1, Southern biotechnology)，再利用 FACS 测定 EGFR 表达水平。其结果如表 4 所示，本发明的人源化抗体 BA03 对多种肺癌，结肠癌，胰腺癌和乳腺癌肿瘤细胞生长有显著抑制作用 (表 4)。

[0155] 表 4 ：BA03 对不同肿瘤细胞的体外活性

[0156]

肿瘤类型	肿瘤细胞株	EGFR 表达水平	% ADCC	肿瘤细胞生长抑制率

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[0157]

肺癌	H460	+	14 %	+
	A549	++	28 %	++
	H292	++	18 %	++
结肠癌	DLD-1	++	27 %	+
	HT-29	++	29 %	+/-
胰腺癌	BXPC3	++	17 %	++
	HPAC	+	15 %	+
乳腺癌	BT474	+/-	3 %	+/-
	SKBr3	+	9 %	+/-
	BT20	+++	22 %	++
	MDA-MB468	+++	18 %	+
	HCC1937	++	21%	++

[0158] 抑制率：+/-5-10%，+10-20%，++20-40%

[0159] 实施例 10 动物药效学实验

[0160] 给 Balb/c 裸鼠在第 0 天时皮下注射人皮肤基底细胞癌 A431 细胞（5×10^6 个细胞/0.1ml/ 每只小鼠）。肿瘤的尺寸用测径器测量，肿瘤的大小使用公式（长 × 宽^2）/2 来计算。当肿瘤大小其平均值达到 100mm^3(61-158mm^3) 时，根据肿瘤大小把荷瘤小鼠随机分配成 3 组。两周内的每个周一、周四腹腔内以 5mg/kg 给药，共计给药 5 次。生理盐水（作为阴性对照）。肿瘤的体积如图 6 所示，生理盐水对照组，肿瘤在三周中很快增长到＞ 1500mm^3，本发明的人源化抗体 BA03 和爱必妥治疗后小鼠肿瘤的增长与对照组相比都被明显抑制（$p < 0.001$，Student's t 检验），但人源化 BA03 抗体具有比爱必妥更好的抗肿瘤活性（$p < 0.05$，Student's t 检验）。

[0161] 尽管本发明的具体实施方式已经得到详细的描述，本领域技术人员将会理解。根据已经公开的所有教导，可以对那些细节进行各种修改和替换，这些改变均在本发明的保护范围之内。本发明的全部范围由所附权利要求及其任何等同物给出。

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[0001]

序列表

<110> 上海津曼特生物科技有限公司
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[0002]

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Asp Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Leu
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50 55 60
Ser Arg Leu Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
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Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
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35 40 45
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50 55 60
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65 70 75 80
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Lys Tyr Ala Ser Glu Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60
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[0005]

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50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Asn Ser Leu Glu Ala
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35 40 45
Lys Tyr Ala Ser Glu Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly
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Ile His Trp Tyr Gln Gln Arg Thr Asn Gly Ser Pro Arg Leu Leu Ile
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Lys Tyr Ala Ser Glu Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly
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[0009]

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[0011]

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260 265 270
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50 55 60
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65 70 75 80
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Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
100 105

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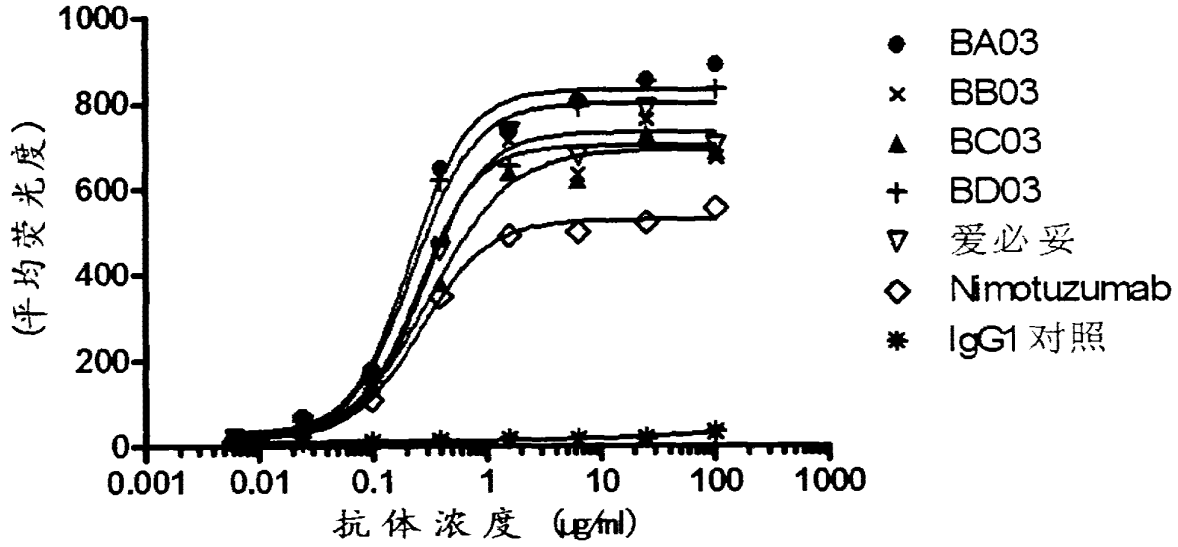


图 1

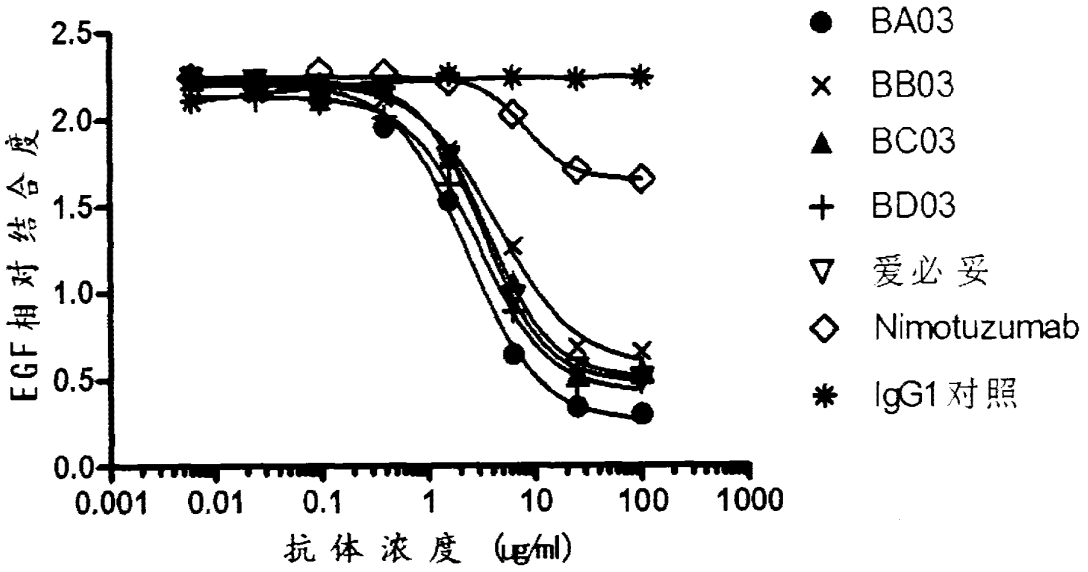


图 2

CSPC Exhibit 1003
Page 233 of 459

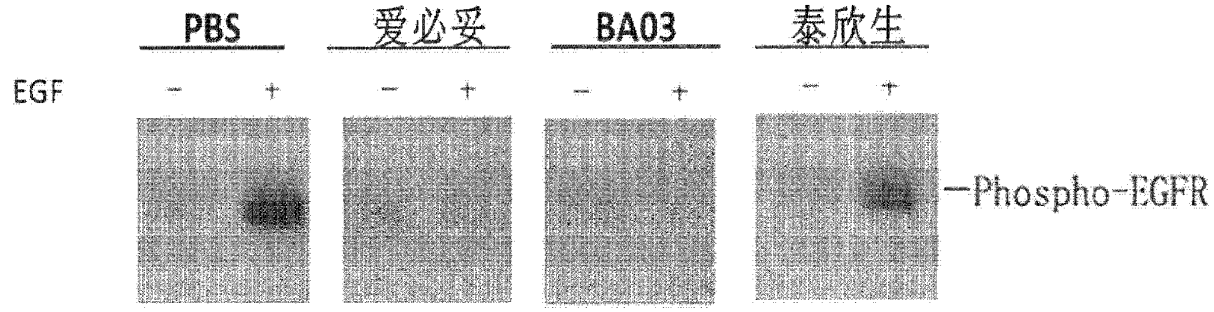


图 3

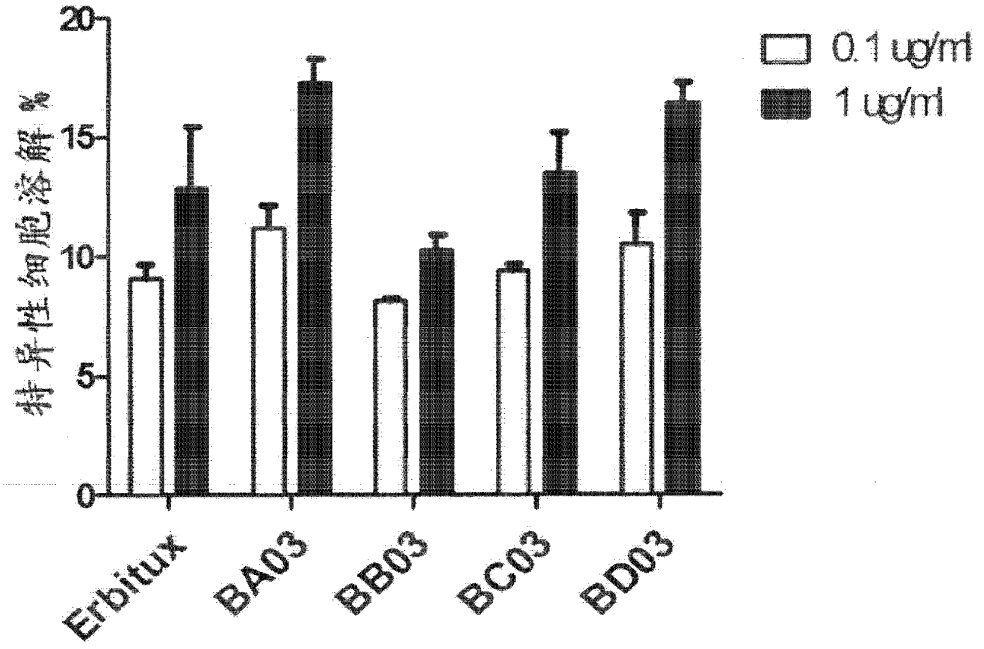


图 4

CSPC Exhibit 1003
Page 234 of 459

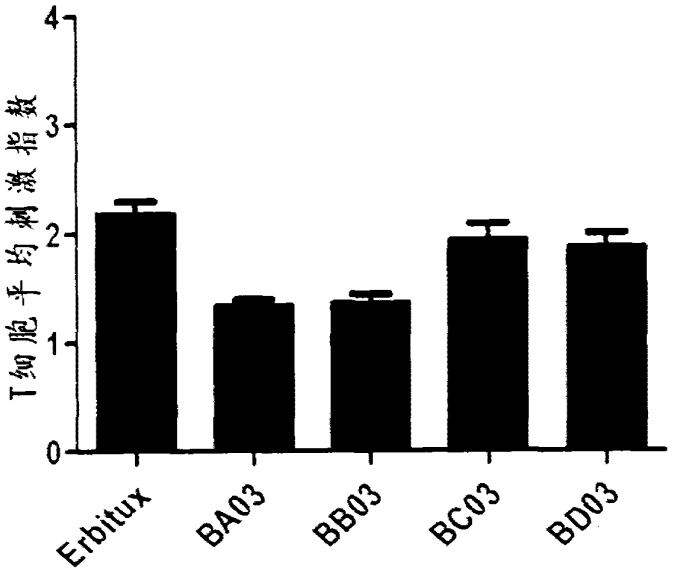


图 5

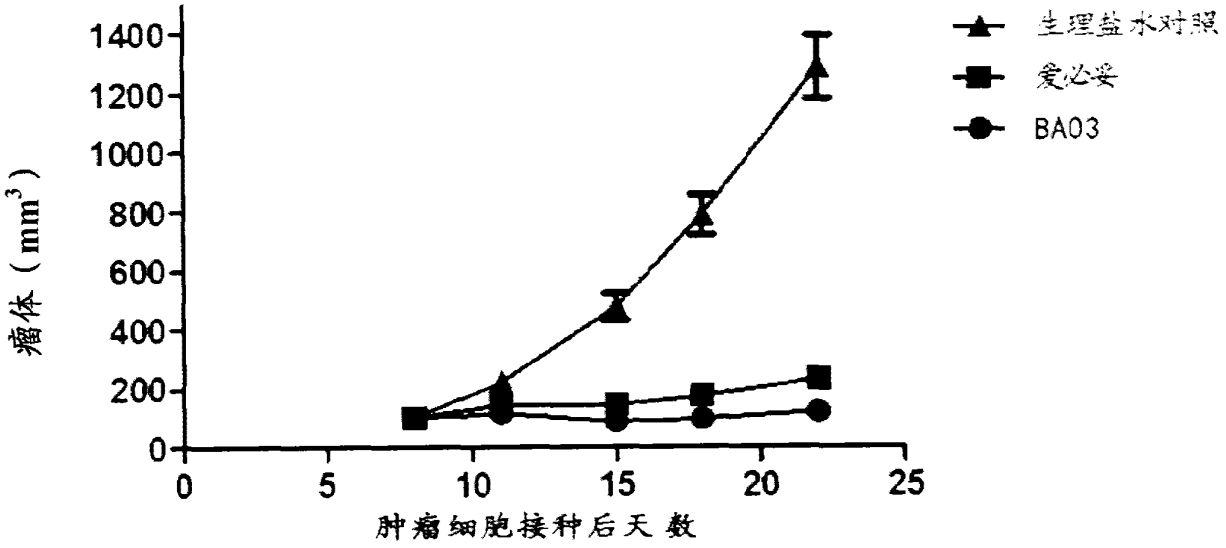


图 6

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Page 235 of 459

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	CCPIT-002
	Application Number	
Title of Invention	Antibody-Drug Conjugate	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 — Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Chaohong		Hu	

Residence Information (Select One) US Residency ● Non US Residency Active US Military Service

City	SHANGHAI	Country of Residence i	CN

Mailing Address of Inventor:

Address 1	SHANGHAI MIRACOGEN INC, SUITE 4E, BUILDING 3,
Address 2	NO.1238 ZHANGJIANG RD, PUDONG DISTRICT

City	SHANGHAI	State/Province	
Postal Code	201203	Country i	CN

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	93726		
Email Address	docket@bozpat.com	Add Email	Remove Email

Application Information:

Title of the Invention	Antibody-Drug Conjugate		
Attorney Docket Number	CCPIT-002	**Small Entity Status Claimed**	☒
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	7	**Suggested Figure for Publication (if any)**	

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	CCPIT-002
	Application Number	
Title of Invention	Antibody-Drug Conjugate	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	● Customer Number	US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	93726		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
	a 371 of international	PCT/CN2016/073844	2016-02-16

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button. Add

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	CCPIT-002
	Application Number	
Title of Invention	Antibody-Drug Conjugate	

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Remove

Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)
201510085038.8	CN	2015-02-17	

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button. Add

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	CCPIT-002
	Application Number	
Title of Invention	Antibody-Drug Conjugate	

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	CCPIT-002
	Application Number	
Title of Invention	Antibody-Drug Conjugate	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1 — Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

Assignee	Legal Representative under 35 U.S.C. 117	Joint Inventor
● Person to whom the inventor is obligated to assign.	Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

Organization Name	SHANGHAI MIRACOGEN INC		
Mailing Address Information For Applicant:			
Address 1	SUITE 4E, BUILDING 3,		
Address 2	NO. 1238 ZHANGJIANG RD, PUDONG DISTRICT		
City	SHANGHAI	**State/Province**	
Country	CN	Postal Code	201203
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button. Add

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	CCPIT-002
	Application Number	
Title of Invention	Antibody-Drug Conjugate	

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

Remove

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country i		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button. Add

Signature:

Remove

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/James S. Keddie/			Date (YYYY-MM-DD)	2017-08-14
First Name	James S.	Last Name	Keddie	Registration Number	48920

Additional Signature may be generated within this form by selecting the Add button. Add

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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	CCPIT-002
	Application Number	
Title of Invention	Antibody-Drug Conjugate	

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1 The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3 A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent CooperationTreaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	Antibody-Drug Conjugate
First Named Inventor/Applicant Name:	Chaohong Hu
Filer:	James Samuel Keddie/Sherry Long
Attorney Docket Number:	CCPIT-002

Filed as Small Entity

Filing Fees for U.S. National Stage under 35 USC 371

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
BASIC NATIONAL STAGE FEE	2631	1	140	140
NATL STAGE SEARCH FEE - REPORT PROVIDED	2642	1	240	240
NATL STAGE EXAM FEE - ALL OTHER CASES	2633	1	360	360
Pages:				
Claims:				
CLAIMS IN EXCESS OF 20	2615	3	40	120
Miscellaneous-Filing:				
Petition:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**860**

Electronic Acknowledgement Receipt

EFS ID:	30071222
Application Number:	15550995
International Application Number:	PCT/CN2016/073844
Confirmation Number:	1813
Title of Invention:	Antibody-Drug Conjugate
First Named Inventor/Applicant Name:	Chaohong Hu
Customer Number:	93726
Filer:	James Samuel Keddie/Sherry Long
Filer Authorized By:	James Samuel Keddie
Attorney Docket Number:	CCPIT-002
Receipt Date:	14-AUG-2017
Filing Date:	
Time Stamp:	17:46:39
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$860
RAM confirmation Number	081517INTEFSW17472800
Deposit Account	
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal of New Application	CCPIT-002_371-Transmittal.pdf	218024 7cfdfc37a2f7dc7c7ba521b0b9f72ec3e67b436e	no	4
Warnings:					
Information:					
2	Documents submitted with 371 Applications	CCPIT-002_Translation_Statement.pdf	25029 6ee536c34d56dd85af1f132ee8599251799a9ed2	no	1
Warnings:					
Information:					
3		CCPIT-002_Application_Eng.pdf	278683 0c13a1abddc0845ee361d7bf38d932494572cc4f	yes	37

Multipart Description/PDF files in .zip description

Document Description	Start	End
Specification	1	29
Claims	30	36
Abstract	37	37

Warnings:					
Information:					
4	Drawings-only black and white line drawings	CCPIT-002_Figures_Eng.pdf	512473 198b0fa9ba68b73ec536a9d498801c2074b9dbd8	no	7
Warnings:					
Information:					
5	Documents submitted with 371 Applications	WO2016131409.pdf	4303620 778b9bbbfd2f131d49cde5ad9e6e23a3422[illegible]	no	43

Warnings:					
Information:					
6		CCPIT-002_Prelim.pdf	138648 949c137f6c84a9a4aa5ae0fc6ac2a094ae79f535	yes	8

Multipart Description/PDF files in .zip description		
Document Description	**Start**	**End**
Preliminary Amendment	1	1
Specification	2	2
Claims	3	7
Applicant Arguments/Remarks Made in an Amendment	8	8

Warnings:					
Information:					
7	Information Disclosure Statement (IDS) Form (SB08)	CCPIT-002_IDS.pdf	1035081 676599d023261d3b9bb4e5579cead48fb6890d94	no	4
Warnings:					
Information:					

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8	Foreign Reference	WO2014152199A1.pdf	4280040 4bfb610a316898c82bd1487fdf4fff9663420dfb	no	95
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Information:					
9	Foreign Reference	CN103772504A.pdf	1898251 cb4fa5a395aec01b5b796b8b0b9aa61185e05af9	no	35
Warnings:					
Information:					

10	Sequence Listing (Text File)	IEC150092PUS_Sequence_Listing.txt	9679 	no	-
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Information:					
11	Application Data Sheet	CCPIT-002_ADS.pdf	1823069 33f9eb7d8fa43ebee9950ccb8981659cc3ee3c53	no	8
Warnings:					
Information:					
12	Fee Worksheet (SB06)	fee-info.pdf	36859 faeb449d4e22aa0b879676c5db850036efb63203	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	14559456	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

第 VIII(iv)栏　声明：发明人资格声明（仅为指定美国目的）

声明必须与规程 214 条的标准语句一致：参见第 VIII 栏、第 VIII(i)到(v)栏的说明(概述)和第 VIII(iv)栏的专门说明。如果不使用本栏，则请求书中不应包括此页。

发明人资格声明 (细则 4.17(iv)和 51 之二.1(a)(iv))
为指定美国目的：

我在此声明我相信我是本申请中要求保护的发明的原始发明人或者共同的原始发明人。

本声明是关于本国际申请的，且是本国际申请的一部分（如果本声明与国际申请一起提出）。

本声明是关于 PCT/CN2016/073844 号国际申请的（如果本声明是根据细则 26 之三提供的）。

我在此声明上述国际申请由我申请或由我授权他人申请。

我在此承认本申请中任何故意作假的声明将依据《美国法典》第 18 篇第 1001 条(United States Code (U.S.C.))受到罚款或不多于五(5)年的监禁或二者并罚的惩罚。

姓名：胡朝红 HU, Chaohong

居所：中国上海市浦东新区张江路 1238 弄 3 号 4E(201203)

邮寄地址：中国上海市浦东新区张江路 1238 弄 3 号 4E(201203)
Suite 4E, Building 3, No 1238 Zhangjiang RD Pudong district, Shanghai 201203, China

发明人的签字：胡朝红　日期：2016-05-06
（该签字必须是发明人的签字，而不是代理人的签字）

姓名：/

/

/

发明人的签字：/　日期：/
（该签字必须是发明人的签字，而不是代理人的签字）

姓名：/

居所：/
(城市，美国的州(如适用)或国家)

邮寄地址：/

/

/

发明人的签字：/　日期：/
（该签字必须是发明人的签字，而不是代理人的签字）

☐ 本声明下转声明续页中“续第 VIII（iv）栏”。

PCT请求书

打印件(原件为电子形式)

0	由受理局填写	
0-1	国际申请号	**PCT/CN2016/073844**
0-2	国际申请日	**2016年 2月 16日 (16.02.2016)**
0-3	受理局名称和"PCT国际申请"	**RO/CN**

0-4	**PCT/RO/101表 PCT请求书**	
0-4-1	软件版本	**CEPCT** 版本 **1.01.00 MT/FOP 20140331/0.20.5.21**
0-5	请求 下列签字人请求按照专利合作条约的规定处理本国际申请	
0-6	申请人指定的受理局	中华人民共和国国家知识产权局 **(RO/CN)**
0-7	申请人或代理人的档案号	**IEC150092PCT**
I	发明名称	抗体药物偶联物
II	申请人	
II-1	该人是	申请人 **(applicant only)**
II-2	是对下列国家的申请人	所有指定国 **(all designated States)**
II-4zh	名称	上海美雅珂生物技术有限责任公司
II-4en	Name:	**SHANGHAI MIRACOGEN INC**
II-5zh	地址	中国上海市 浦东新区张江路**1238**弄**3**号**4E** **201203**
II-5en	Address	**SUITE 4E, BUILDING 3, NO. 1238 ZHANGJIANG RD,** **PUDONG DISTRICT** **Shanghai 201203** **China**
II-6	国籍	中国 **CN**
II-7	居所	中国 **CN**
II-8	电话号码	
II-9	传真号码	
II-11	申请人在专利局的登记号	
III-1	申请人和/或发明人	
III-1-1	该人是	发明人 **(inventor only)**
III-1-3	对下列国家的发明人	
III-1-4zh	姓名:	胡 朝红
III-1-4en	Name (LAST, First):	**HU, Chaohong**
III-1-5zh	地址	中国上海市 浦东新区张江路**1238**弄**3**号**4E** **201203**
III-1-5en	Address	**SUITE 4E, BUILDING 3, NO. 1238 ZHANGJIANG RD,** **PUDONG DISTRICT** **Shanghai 201203** **China**

PCT请求书

打印件(原件为电子形式)

IV-1	代理人,共同代表或通信地址 下列人员被委托/已经被委托为代表申请人在主管国际单位办理事务的	代理人 **(agent)**
IV-1-1zh	名称	中国国际贸易促进委员会专利商标事务所
IV-1-1en	Name:	**CCPIT PATENT AND TRADEMARK LAW OFFICE**
IV-1-2zh	地址	中国北京市 西城区阜成门外大街**2**号万通新世界广场**8**层 **100037**
IV-1-2en	Address:	**8th Floor, Vantone New World Plaza, 2 Fuchengmenwai Street, Xicheng District** **Beijing 100037** **China**
IV-1-3	电话号码	**861066412345**
IV-1-4	传真号码	**861066415678**
IV-1-5	电子邮址	**pct@ccpit-patent.com.cn**
IV-1-5(a)	电子邮件授权 授权受理局、国际检索单位、国际局和国际初步审查单位，如果其愿意，使用本电子邮件地址发送有关本国际申请的通知书：	作为随后纸件通知书的预送本
IV-1-6	代理人登记号	**11038**
V	国家的指定	
V-1	根据细则**4.9(a)**，提交本请求书即为，指定在国际申请日受**PCT**约束的所有成员国,以要求获得可以获得的所有保护类型，适用情况下，要求获得地区专利和国家专利。	
V-2	**V-2**栏只可用于（不可悔改地）排除对有关国家的指定，如果本国际申请在提交时或者在根据细则**26**之二**.1**的后续程序中，第**VI**栏包含有对在该有关国家提出的在先国家申请的优先权要求，以避免被要求优先权的该在先国家申请因国家法律而停止效力。	
VI-1	要求在先国家申请的优先权	
VI-1-1	申请日	**2015**年 **2**月 **17**日 **(17.02.2015)**
VI-1-2	号码	**201510085038.8**
VI-1-3	国家	中国 **CN**
VI-2	优先权文件请求 请国际局从数字图书馆获取上面指明的在先申请的证明副本，查询码为:	**VI-1** 查询码**: A8BE**
VII-1	选定的国际检索单位	中华人民共和国国家知识产权局 **(ISA/CN)**
VIII	声明	声明数目
VIII-1	关于发明人身份的声明	-
VIII-2	关于申请人在国际申请日有权申请和被授予专利的声明	-
VIII-3	关于申请人在国际申请日有权要求在先申请的优先权的说明	-
VIII-4	发明人资格声明(仅为指定美国目的)	-
VIII-5	关于不影响新颖性的公开或缺乏新颖性的例外的声明	-

PCT请求书

打印件(原件为电子形式)

IX	清单	页数	附以电子文档
IX-1	请求书(包括声明页)	**3**	✓
IX-2	说明书	**24**	✓
IX-3	权利要求	**5**	✓
IX-4	摘要	**1**	✓
IX-5	附图	**7**	✓
IX-6a	说明书序列表部分(也将用于国际检索的目的)	**-**	✓
IX-7	共计	**40**	
	附件	附以纸件	附以电子文档
IX-8	费用计算页	**-**	✓
IX-18	PCT-SAFE 物理载体	**-**	**-**
IX-20	应与摘要一起公布的附图图号		
IX-21	国际申请所用语言	中文	
X-1	申请人，代理人或共同代表的签字	/刘向昕/	
X-1-1	名称	中国国际贸易促进委员会专利商标事务所	
X-1-2	签字人姓名	中国国际贸易促进委员会专利商标事务所	
X-1-3	还要注明此人是以什么名义签字的	代理人	

由受理局填写

10-1	据称的国际申请文件的实际收到日期	**2016年 2月 16日 (16.02.2016)**
10-2	附图:	
10-2-1	收到	
10-2-2	未收到	
10-3	由于随后在期限内收到补充国际申请的文件或附图,更改的实际收到日期	
10-4	在期限内收到根据**PCT**第**11(2)**条所进行的改正的日期	
10-5	国际检索单位	**ISA/CN**
10-6	检索本的传送推迟到缴纳检索费后	

由国际局填写

11-1	国际局收到登记本的日期	

专 利 合 作 条 约

发信人：国际检索单位

收信人：
100037
中国北京市西城区阜成门外大街2号万通新世界广场8层

中国国际贸易促进委员会专利商标事务所

PCT

国际检索单位书面意见

(PCT细则43之二 .1)

发文日 (年/月/日)	2016年 5月 18日
申请人或代理人的档案号	关于后续行为
IEC150092PCT	见下面第2段

国际申请号	国际申请日 (年/月/日)	优先权日 (年/月/日)
PCT/CN2016/073844	2016年 2月 16日	2015年 2月 17日

国际专利分类（IPC）或国家分类及IPC
A61K 47/48(2006.01)i; A61K 45/00(2006.01)i; A61P 35/00(2006.01)i

申请人
上海美雅珂生物技术有限责任公司

1. 本意见包括关于下列各项标明的内容：

- [x] 第I栏 意见的基础
- [ ] 第II栏 优先权
- [x] 第III栏 不做出关于新颖性、创造性和工业实用性的意见
- [ ] 第IV栏 缺乏发明的单一性
- [x] 第V栏 按照细则43之二.1(a)(i)关于新颖性、创造性或工业实用性的推断性声明；支持这种声明的引证和解释
- [ ] 第VI栏 某些引用的文件
- [x] 第VII栏 国际申请中的某些缺陷
- [x] 第VIII栏 对国际申请的某些意见

2. 后续行为

如果提出初步审查要求书，本次意见将被视为国际初步审查单位(IPEA)的一次书面意见，除非申请人选择的国际初步审查单位非本机构，而且所选国际初步审查单位已按照细则66.1之二(b)通知国际局将不考虑国际检索单位的书面意见时例外。

如本书面意见被视为国际初步审查单位的书面意见，则请申请人在自PCT/ISA/220表发文日起3个月或自优先权日起22个月内（以后届满者为准）向国际初步审查单位提交书面答复并提交修改（如适用）。

进一步的选择参见PCT/ISA/220表。

ISA/CN的名称和邮寄地址	完成本意见的日期	受权官员
中华人民共和国国家知识产权局 中国北京市海淀区蓟门桥西土城路6号 100088	2016年 5月 13日	何睿
传真号 （86－10）62019451		电话号码 (86-10)62411106

表 PCT/ISA/237 （扉页） (2011年7月)

国际检索单位书面意见

国际申请号
PCT/CN2016/073844

第I栏 意见的基础

1. 关于语言，本意见的制定基于：
   - [x] 国际申请提交时使用的语言。
   - [ ] 该国际申请的_____语言译文，为了国际检索的目的提供该种语言的译文(细则12.3(a)和23.1(b))。

2. [ ] 本意见的制定考虑了本单位许可或被通知的根据细则91所做出的明显错误更正（细则 43之二1（a））。

3. 关于国际申请中所公开的任何对要求保护的发明必要的核苷酸和/或氨基酸序列，本意见是在下列基础上制定的：

   a. (提交提供)
      - [ ] 纸件形式
      - [x] 电子形式

   b. (提交时间)
      - [x] 含在申请提交时的国际申请中
      - [ ] 以电子形式与国际申请一起提交
      - [ ] 为检索之用随后提交本单位

4. [ ] 另外，在提交/提供了多个版本或副本的序列表的情况下，提供了随后或附加副本中的信息与申请时提交的申请中的信息相同或未超出申请时提交的申请中的信息范围（如适用）的所需声明。

5. 补充意见：

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第III栏　　不做出关于新颖性、创造性和工业实用性的意见

对于

☐ 整个国际申请

☑ 权利要求 参见以下权利要求

没有审查要求保护的发明看来是否具备新颖性、创造性(非显而易见性)，或者工业实用性的问题，

因为：

☑ 该国际申请，或者该权利要求 23-28 涉及不要求国际检索的主题(具体说明）：

[1] PCT细则39.1（iv）——处置人体或者动物体的外科手术方法或治疗方法，以及诊断方法。

☐ 说明书、权利要求书或者附图(下面特别指明的部分)或者所述权利要求_____不清楚，以致不能够形成任何有意义的意见(具体说明)：

☐ 权利要求书或所述权利要求_____没有得到说明书的充分支持，以致不能形成任何有意义的意见（具体说明）：

☑ 对所述权利要求 23-28 没有做出国际检索报告。

☐ 没有序列表，无法做出有意义的意见；申请人没有在规定的期限内：

☐ 没有提供符合行政规程附件C中所规定的标准的纸件形式的序列表，并且国际检索单位也未获得式和方式可以被接受的序列表。

☐ 没有提供符合行政规程附件c中所规定的标准的电子形式的序列表，并且国际检索单位也未获得式和方式可以被接受的序列表。

☐ 在答复根据细则13之三.1(a)或（b)的通知提交序列表时，没有缴纳所要求的后提交费。

☐ 详情见补充栏

国际检索单位书面意见

国际申请号
PCT/CN2016/073844

第V栏　　按细则43之二.1（a）（i）关于新颖性、创造性或工业实用性的推测性声明；支持这种声明的引证和解释

1. 声明

新颖性 (N)	权利要求	2-4、21、22、33和34	是
	权利要求	1、5-20和29-32	否
创造性 (IS)	权利要求	无	是
	权利要求	1-22和29-34	否
工业实用性 (IA)	权利要求	1-22和29-34	是
	权利要求	无	否

2. 引证和解释：

[1] 由国际检索单位作出的关于新颖性、创造性或工业实用性的书面意见是基于可以被合理预期要求保护的主题，详情可见第Ⅷ栏。

[2] 引用以下的对比文件

[3] D1：WO 2014152199A1　　25.9月2014（25.09.2014）

[4] D2：CN 103772504A　　07.5月2014（07.05.2014）

[5] 新颖性和创造性（PCT条约第33条第2和3款）

[6] D1（参见摘要，权利要求1-50，说明书第25页第1行至第36页第3行，图1）公开了一种抗体药物缀合物，所述抗体为抗EGFR抗体，所述药物可以为MMAE，并公开了包含所述抗体药物缀合物的组合物和所述抗体药物缀合物的用途。

[7] D2（参见权利要求1-13，实施例1-10）公开了一种抗EGFR抗体BA03，其可变区和恒定区的氨基酸序列与本申请的抗体相同。

[8] D1公开的缀合物中的药物属于细胞毒剂，药物与抗EGFR抗体通过接头共价连接。因此，权利要求1不具备新颖性。

[9] D2公开了一种抗EGFR抗体，其可变区序列与权利要求2-4中限定的序列相同。因此，在D1的基础上结合D2得到权利要求2-4的技术方案，对本领域技术人员来说是显而易见的。权利要求2-4不具备创造性。

[10] D1公开的抗EGFR抗体的重链恒定区与SEQ ID NO:3有3个氨基酸不同，轻链恒定区与SEQ ID NO:4有一个氨基酸不同。当其引用的权利要求不具备新颖性/创造性时，权利要求5-9也不具备新颖性/创造性。

[11] D1公开的抗体药物缀合物的结构落入式Ⅰ的范围内。当其引用的权利要求不具备新颖性/创造性时，权利要求10也不具备新颖性/创造性。

[12] D1公开所述药物可以为MMAE、MMAF等。当其引用的权利要求不具备新颖性/创造性时，权利要求11和12也不具备新颖性/创造性。

[13] D1公开所述接头可为可切割接头，可为MC、PAB、SMCC、SPP、缬氨酸-瓜氨酸等。当其引用的权利要求不具备新颖性/创造性时，权利要求13和14也不具备新颖性/创造性。

[14] D1公开了一种具体的MMAE连接到抗体的连接方式，其与权利要求15限定的结构相同，药物个数可以为2或4。当其引用的权利要求不具备新颖性/创造性时，权利要求15也不具备新颖性/创造性。

[15] D1公开了包含所述抗体药物缀合物的组合物，并公开了所述抗体药物缀合物可用于诊断和治疗EGFR相关疾病，可用于诊断和治疗癌症，例如肺癌、结肠癌、头颈部癌、乳腺癌等。当其引用的权利要求不具备新颖性/创造性时，权利要求16-20、29-32也不具备新颖性/创造性。

[16] 在D1的启示下，本领域技术人员能够预期所述抗EGFR抗体-药物缀合物可以用于治疗KRAS基因突变的肿瘤和BRAF基因突变的肿瘤。因此，权利要求21、22、33、34不具备创造性。

[17] 工业实用性（PCT条约第33条第4款）

[18] 权利要求1-22、29-34所述发明可在生物医药技术领域制造和使用，因此符合工业实用性的规定。

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第VII栏　　国际申请中的某些缺陷

国际申请具有下列形式或内容缺陷：

[1] 由于多项从属权利要求8、10、11、15、29、31引用了在前的多项从属权利要求，所述权利要求不满足PCT细则6.4（a）的规定。

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第VIII栏　　对国际申请的某些意见

就权利要求、说明书和附图的清楚性或者就权利要求是否得到说明书的充分支持提出以下意见：

[1] 权利要求2-5、8中使用了“突变体”，本领域技术人员无法确定其范围。因此，所述权利要求不清楚，不符合PCT条约第6条的规定。

[2] 为了便于检索和出具书面意见，将权利要求2-5、8的技术方案预期为删除“突变体”后的技术方案。

[3] 权利要求12中使用了“等”，这导致权利要求12不清楚，不符合PCT条约第6条的规定。

[4] 为了便于检索和出具书面意见，将权利要求12的技术方案预期为删除“等”后的技术方案。

CSPC Exhibit 1003

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(DAS)

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ORGANIZATION

CERTIFICATE OF AVAILABILITY OF A CERTIFIED PATENT DOCUMENT IN A DIGITAL LIBRARY

The International Bureau certifies that a copy of the patent application indicated below has been available to the WIPO Digital Access Service since the date of availability indicated, and that the patent application has been available to the indicated Office(s) as of the date specified following the relevant Office code:

Document details: Country/Office: CN
Filing date: 17 Feb 2015 (17.02.2015)
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证　明

本证明之附件是向本局提交的下列专利申请文件副本。

申　　请　　号：201510085038.8

申　请　类　型：发明专利

发明 创造 名称：抗体药物偶联物

申　　请　　日：2015 年 02 月 17 日

申　　请　　人：上海美雅珂生物技术有限责任公司

发明人或设计人：胡朝红

局长

申长雨

2016 年 01 月 27 日

权 利 要 求 书

1. 抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其包含与细胞毒剂共价连接的抗表皮生长因子受体抗体。

2. 权利要求 1 的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的抗表皮生长因子受体抗体包括重链和轻链，其中重链可变区 CDR1、CDR2、CDR3 分别包含如 SEQ ID NO: 5～7 所示的序列或其突变体，轻链可变区 CDR1、CDR2、CDR3 分别包含如 SEQ ID NO: 12～14 所示的序列或其突变体。

3. 权利要求 2 的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的抗表皮生长因子受体抗体重链可变区 FR1、FR2、FR3、FR4 区分别包含如 SEQ ID NO: 8～11 所示的序列或其突变体。

4. 权利要求 2 的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的抗表皮生长因子受体抗体轻链可变区 FR1、FR2、FR3、FR4 区分别包含如 SEQ ID NO: 15～18 所示的序列或其突变体。

5. 权利要求 1-4 任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的抗表皮生长因子受体抗体的重链恒定区选自人源性 IgG、IgM、IgA、IgD、IgA 恒定区或上述恒定区的突变体。

6. 权利要求 5 的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述 IgG 选自 IgG1、IgG2、IgG3 和 IgG4。

7. 权利要求 5 的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的抗表皮生长因子受体抗体重链恒定区的氨基酸序列包含如 SEQ ID NO: 3 所示的序列，或者包含与 SEQ ID NO: 3 所示序列的同一性大于 70%，例如大于 75%、80%、85%、90%、95%、99%的序列。

8. 权利要求 1-7 任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的抗表皮生长因子受体抗体的轻链恒定区为人源性 lambda 恒定区、kappa 恒定区、或上述恒定区的突变体。

9. 权利要求 8 的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的抗表皮生长因子受体抗体轻链恒定区的氨基酸序列包含如 SEQ ID NO: 4 所示的序列，或者包含与 SEQ ID NO: 4 所示序列的同一性大于 70%，例如大于 75%、80%、85%、90%、95%、99%的序列。

10. 权利要求 1-9 任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其具有式Ⅰ：$Ab-(L-D)_p$ 所示的结构，其中：

Ab 代表抗表皮生长因子受体抗体；

L 代表接头；

D 代表细胞毒剂；

p 代表 1-9，例如 2-6，例如 3-5。

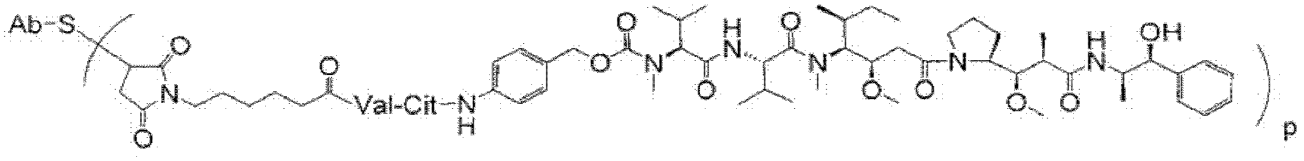
11. 权利要求1-10任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的细胞毒剂选自化疗药物、毒素、放射性同位素、细胞因子、抗生素、酶、纳米颗粒或生物活性肽。

12. 权利要求11的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的细胞毒剂选自Monomethyl auristatin E（MMAE）、 Monomethyl auristatin F（MMAF）、 美登木素生物碱（例如Maytansine DM1、Maytansine DM4）、卡奇霉素(calicheamicin)、 duocarmycin MGBA、阿霉素(doxorubicin)、蓖麻毒素、白喉毒素等毒素、I131、白介素类、肿瘤坏死因子、趋化因子、纳米颗粒等。

13. 权利要求 10 的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的接头为可切割的或不可切割的。

14. 权利要求 13 的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的接头选自 6-马来酰亚氨基己酰基(MC)、马来酰亚氨基丙酰基(MP)、缬氨酸-瓜氨酸(val-cit)、丙氨酸-苯丙氨酸(ala-phe)、对氨基苄氧羰基(PAB)、N-琥珀酰亚氨基 4-(2-吡啶基硫代)戊酸酯(SPP)、N-琥珀酰亚氨基 4-(N-马来酰亚氨基甲基)-环己烷-1-羧酸酯(SMCC)、N-琥珀酰亚氨基(4-碘-乙酰基)氨基苯甲酸酯(SIAB)、和 6-马来酰亚氨基己酰基-缬氨酸-瓜氨酸-对氨基苄氧羰基(MC-vc-PAB)。

15. 权利要求 1-14 任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其为：



其中Ab为抗表皮生长因子受体抗体，p为1-9，例如2-6，例如3-5。

16. 组合物，其含有权利要求1-15任一项的抗体药物偶联物、其药

学上可接受的盐、溶剂合物或所述盐的溶剂合物，任选地，还含有至少一种药学上可接受的载体、稀释剂或赋形剂。

17. 权利要求1-15任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物在制备预防和/或治疗与表皮生长因子受体（EGFR）相关的疾病的药物中的用途。

18. 权利要求16的用途，其中所述的与表皮生长因子受体（EGFR）相关的疾病为与EGFR相关的肿瘤，例如与EGFR高表达相关的肿瘤，例如选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

19. 权利要求1-15任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物在制备抑制肿瘤血管生成、延缓肿瘤进展、抑制肿瘤生长、抑制肿瘤细胞增殖的药物中的用途。

20. 权利要求19的用途，其中所述的肿瘤选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

2015100850388

说　　明　　书

抗体药物偶联物

技术领域

本发明涉及抗体药物偶联物，具体涉及抗表皮生长因子受体抗体药物偶联物。本发明还涉及包含该抗体药物偶联物的组合物，以及该抗体药物偶联物的医药用途。

背景技术

表皮生长因子受体 (Epidermal Growth Factor Receptor，EGFR，也称为 HER1， c-ErbB1) 是表皮生长因子家族的细胞表面受体，是由 1186 个氨基酸残基构成，分子量为 170kD 的一种跨膜糖蛋白 (Jorissen RN，Walker F，Pouliot N，et al.Epidermal growth factor receptor: mechanisms of activation and signaling.Exp Cell Res，2003；284：31-53)。EGFR 属于Ⅰ型酪氨酸激酶受体 ErbB 亚族 (ErbB 1-4)，具有酪氨酸激酶的活性。EGFR 稳定的表达于许多上皮组织，包括皮肤和毛囊。表皮生长因子受体异常表达或因受体突变而活化会导致癌变。有很多实体瘤发现高表达表皮生长因子受体，如直结肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌和食管癌等 (Olayioye MA，Neve RM，Lane HA，et al.TheEerbB Signaling network：receptor heterodimerzation in development and cancer.The EMBO J，2000；19：3159-3167)。转化生长因子 α 和表皮生长因子等生长因子是表皮生长因子受体的内源性配体。这些配体与表皮生长因子受体结合，激活受体胞内酪氨酸蛋白激酶的活性，启动下游多条信号转导途径，从而调节正常细胞的生长和分化，增加肿瘤细胞的侵袭力、促进血管生成、抑制肿瘤细胞的凋亡 (Ciardiello F，Tortora G.A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor.Clin Cancer Res，2001；7：2958-2970)。表皮生长因子受体在肿瘤中的高度表达及其在肿瘤细

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胞生长、分化中起重要作用的这些特点，使表皮生长因子受体成为具有良好前景的肿瘤治疗靶点。

目前市场上已有两个抗表皮生长因子受体的治疗性抗体，一个是人鼠嵌合抗体 C225 抗体（爱必妥，Erbitux 或 Cetuximab，ImClone（现在 Eli Lilly）公司），与表皮生长因子受体有特异亲和性，可阻断 EGF 和 TGF 等配体与表皮生长因子受体的结合，抑制其磷酸化和下游信号传导，从而抑制肿瘤细胞的生长，诱导凋亡，减少基质金属蛋白酶和血管上皮生长因子的产生。2004 年美国 FDA 批准爱必妥治疗结直肠癌，2006 批准治疗头颈部癌，目前有更多临床试验用于其它肿瘤适应症。在临床上爱必妥和伊立替康合用治疗结直肠癌有效率（ORR）为 23%，与氟嘧啶等化药合用治疗头颈癌有效率为 13%-30%。因为是人鼠嵌合抗体，爱必妥抗体在临床试验中有 3.7%的病人产生了抗体反应。

另一个抗表皮生长因子受体的治疗性抗体是帕尼单抗（Vectibix，panitumumab，Amgen 公司），是采用转基因小鼠技术制备的全人化单克隆抗体，无鼠源蛋白序列，靶向作用于表皮生长因子受体（EGFR），2006 年 9 月被 FDA 批准上市，与氟嘧啶、奥沙利铂和伊立替康合用或在化疗后用于治疗 EGFR 阳性的转移性直结肠癌。2006 年 FDA 批准其单药治疗化疗耐受的转移性结直肠癌(mCRC)。然而帕尼单抗是 IgG2 亚型抗体，与 IgG1 相比，IgG2 的 CDC 活性及 ADCC 等生物学活性明显减低；另外，IgG2 亚型抗体稳定性较差，这可能是临床效果上全人抗体帕尼单抗与嵌合抗体爱必妥相比没有明显优势的主要原因。在临床上治疗结直肠癌的总体生存率（OR）才达到 8%，非进展生存期只延长了 3.6 个月。

目前大量的临床数据显示爱必妥单抗和帕尼单抗只对 EGFR 表达的 KRAS 野生型（KRAS wild type）有疗效，而对 KRAS 突变体没有肿瘤生长抑制活性，因此，美国临床肿瘤协会发表的指导原则中明确指出抗 EGFR 单抗药物只适用于 KRAS 野生型的结肠癌病人（Allegra CJ, Jessup JM, Somerield MR, Hamilton SR, Hammond EH, Hayes DF,

et al. American Soceitey of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor monoclonal antibody therapy. J. Clin Oncol. 2009; 27:2091-2096; Bardelli A, Siena S. Molecular mechanisms of resistance to cetuximab and panitumumab in colorectal cancer. J Clin Oncol. 2010; 28:1254-1261）。

因此，本领域亟需具有更高生物学活性的抗人源化抗表皮生长因子受体的抗体类药物，尤其是对 KRAS 突变体有疗效的药物，例如抗体药物偶联物以进一步提高疗效，降低副作用。

由于单抗癌症药物的引领，近年来在欧美快速发展的抗体药物偶联物（antibody drug conjugate， ADC）成为目前世界最前沿的医药技术。

抗体药物偶联物一般由三个部分组成:

1. 专一性结合靶点的单抗;
2. 细胞毒性的小分子化学药物;
3. 链接小分子药物和单抗的链接体，也叫接头。

抗体药物偶联物利用单抗的靶点专一性和化药的细胞毒性来杀死肿瘤细胞。它的作用机制是：（1）抗体药物偶联物利用单抗特异地结合到肿瘤细胞上的靶点；（2）抗体药物偶联物和靶点的复合物转移进入细胞内；（3）抗体药物偶联物在细胞内降解，释放细胞毒的化药；（4）细胞毒的化药杀死肿瘤细胞。

抗体药物偶联物的作用机制看似简单， 但是一个抗体药物偶联物是否能成为安全有效的药物是非常复杂和不可预测的，依赖多种因素，例如:

1) 靶点的特性： 靶点是否能内吞噬 （internalize），靶点的表达水平，靶点是否在癌细胞和正常细胞中有足够的表达水平差异，靶点是否会脱落细胞外部分（extracellular domain, ECD）到血液中;

2) 单抗的特性： 单抗对靶点的特异性是否足够好 （与其它蛋白无交叉反应）， 单抗的稳定性如何，单抗和靶点的复合物是否能内

吞噬入细胞;

3) 小分子化药的特性: 化药的细胞毒性是否足够强,化药在血液里的稳定性,化药成为 ADC 后在体内的代谢产物的毒性;

4) 接头的特性: 接头是可切割的 (cleavable)还是不可切割的 (non-cleavable),接头在血液里的稳定性;

5) ADC 的特性: ADC 和靶点的复合物是否能内吞噬,ADC 在血液里的稳定性,ADC 上用什么链接体和链接几个化药,ADC 杀癌细胞的活性和生物毒性。

可以看出,ADC 药物的研发需要大量的实验摸索和验证,其安全性和有效性是无法在实验前预测的。

发明内容

本发明的发明人通过大量实验和创造性劳动,制备得到了抗表皮生长因子受体抗体药物偶联物,并证实其具有良好的生物学活性,由此完成了本发明。

本发明第一方面涉及抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物,其包含与细胞毒剂共价连接的抗表皮生长因子受体抗体。

在本发明的一个实施方案中,其中所述的抗表皮生长因子受体抗体包括重链和轻链,其中重链可变区 CDR1、CDR2、CDR3 分别包含如 SEQ ID NO: 5~7 所示的序列或其突变体,轻链可变区 CDR1、CDR2、CDR3 分别包含如 SEQ ID NO: 12~14 所示的序列或其突变体。

在本发明的实施方案中,其中所述的抗表皮生长因子受体抗体轻链可变区 CDR1、CDR2、CDR3 区分别包含如 SEQ ID NO: 12~14 所示的序列或者与 SEQ ID NO: 12~14 所示的序列的同一性大于 70%,例如大于 75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%的序列,例如具有 3 个、2 个或 1 个突变、缺失或添加氨基酸的序列。

在本发明的实施方案中，其中所述的抗表皮生长因子受体抗体重链可变区 CDR1、CDR2、CDR3 区分别包含如 SEQ ID NO: 5～7 所示的序列或者与 SEQ ID NO: 5～7 所示的序列的同一性大于 70%，例如大于 75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%的序列，例如具有 3 个、2 个或 1 个突变、缺失或添加氨基酸的序列。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体重链可变区 FR1、FR2、FR3、FR4 区分别包含如 SEQ ID NO: 8～11 所示的序列或其突变体。

在本发明的实施方案中，其中所述的抗表皮生长因子受体抗体重链可变区 FR1、FR2、FR3、FR4 区分别包含如 SEQ ID NO: 8～11 所示的序列，或者包含与上述序列的同一性大于 70%，例如大于 75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%的序列。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体轻链可变区 FR1、FR2、FR3、FR4 区分别包含如 SEQ ID NO: 15～18 所示的序列或其突变体。

在本发明的实施方案中，其中所述的抗表皮生长因子受体抗体轻链可变区 FR1、FR2、FR3、FR4 区分别包含如 SEQ ID NO: 15～18 所示的序列，或者包含与上述序列的同一性大于 70%，例如大于 75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%的序列。

在本发明的一个实施方案中，所述抗表皮生长因子受体抗体的重链可变区的序列如 SEQ ID NO: 1 所示。

在本发明的一个实施方案中，所述抗表皮生长因子受体抗体的轻链可变区的序列如 SEQ ID NO: 2 所示。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体的重链恒定区选自人源性 IgG、IgM、IgA、IgD、IgA 恒定区或上述恒定区的突变体。

在本发明的一个实施方案中，其中所述 IgG 选自 IgG1、IgG2、IgG3 和 IgG4。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体重链恒定区的氨基酸序列包含如 SEQ ID NO: 3 所示的序列，或者包含与 SEQ ID NO: 3 所示序列的同一性大于 70%，例如大于 75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%的序列。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体的轻链恒定区为人源性 lambda 恒定区、kappa 恒定区、或上述恒定区的突变体。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体轻链恒定区的氨基酸序列包含如 SEQ ID NO: 4 所示的序列，或者包含与 SEQ ID NO: 4 所示序列的同一性大于 70%，例如大于 75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%的序列。

在本发明的一个实施方案中，其具有式Ⅰ：$Ab-(L-D)_p$ 所示的结构，其中：

Ab 代表抗表皮生长因子受体抗体；

L 代表接头；

D 代表细胞毒剂；

p 代表 1-8，例如 2-6，例如 3-5。

在本发明的一个实施方案中，其中所述的细胞毒剂选自化疗药物、毒素（例如细菌、真菌、植物、或动物源性的酶活性毒素或其片段）、放射性同位素、细胞因子、抗生素、酶、纳米颗粒或生物活性肽。

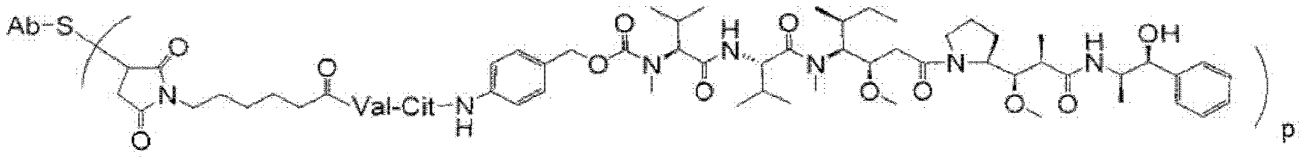
在本发明的一个实施方案中，其中所述的细胞毒剂选自 Monomethyl auristatin E（MMAE）、 Monomethyl auristatin F（MMAF）、 美登木素生物碱（例如Maytansine DM1、Maytansine DM4）、卡奇霉素(calicheamicin)、 duocarmycin MGBA、阿霉素(doxorubicin)、蓖麻毒素、白喉毒素等毒素、I131、白介素类、肿瘤坏

死因子、趋化因子、纳米颗粒等。

在本发明的一个实施方案中，其中所述的接头为可切割的或不可切割的。

在本发明的一个实施方案中，其中所述的接头选自 6-马来酰亚氨基己酰基(MC)、马来酰亚氨基丙酰基(MP)、缬氨酸-瓜氨酸(val-cit)、丙氨酸-苯丙氨酸(ala-phe)、对氨基苄氧羰基(PAB)、N-琥珀酰亚氨基 4-(2-吡啶基硫代)戊酸酯(SPP)、N-琥珀酰亚氨基 4-(N-马来酰亚氨基甲基)-环己烷-1-羧酸酯(SMCC)、N-琥珀酰亚氨基(4-碘-乙酰基)氨基苯甲酸酯(SIAB)、和 6-马来酰亚氨基己酰基-缬氨酸-瓜氨酸-对氨基苄氧羰基(MC-vc-PAB)。

在本发明的一个具体实施方案中，其为:



其中Ab为抗表皮生长因子受体抗体，p为1-8，例如2-6，例如3-5。

本发明第二方面涉及组合物（例如药物组合物），其含有本发明第一方面任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，任选地，还含有至少一种药学上可接受的载体、稀释剂或赋形剂。

在本发明的一个实施方案中，所述组合物中还包含已知的用于治疗肿瘤的化疗药物，所述化疗药物例如为阿霉素(Adriamycin)、环磷酰胺和紫杉烷类[紫杉醇(Taxol)和多西他赛(Taxotere)]、卡培他滨(Xeloda)、吉西他滨(Gemzar)、长春瑞滨(Navelbine)、他莫昔芬、芳香酶抑制剂(瑞宁得、弗隆、阿诺新)、5-FU 加亚叶酸、伊立替康(camptosar)、奥沙利铂、顺铂、卡铂、雌莫司汀、米托蒽醌(Novantrone)、泼尼松、长春新碱(Oncovin)等，或它们的组合。

本发明还涉及本发明第一方面任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物在制备预防和/或治疗与表面生长因子受体（EGFR）相关的疾病的药物中的用途。

在本发明的实施方案中，其中所述的与表面生长因子受体（EGFR）相关的疾病为与EGFR相关的肿瘤，例如与EGFR高表达相关的肿瘤，例如选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

本发明还涉及本发明第一方面任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物在制备抑制肿瘤血管生成、延缓肿瘤进展、抑制肿瘤生长、抑制肿瘤细胞增殖的药物中的用途。

在本发明的实施方案中，其中所述的肿瘤选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

本发明还涉及预防和/或治疗与表皮生长因子受体（EGFR）相关的疾病的方法，所述方法包括给予有需要的受试者预防和/或治疗有效量的本发明第一方面任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物。

本发明还涉及抑制肿瘤血管生成、延缓肿瘤进展、抑制肿瘤生长、抑制肿瘤细胞增殖的方法，所述方法包括给予有需要的受试者预防和/或治疗有效量的本发明第一方面任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物。

在本发明的实施方案中，其中所述的与表皮生长因子受体（EGFR）相关的疾病为与EGFR相关的肿瘤，例如与EGFR高表达相关的肿瘤，例如选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

本发明的抗表皮生长因子受体抗体药物偶联物、其药学上可接受

的盐、溶剂合物或所述盐的溶剂合物在体内和体外都具有良好的抑制肿瘤细胞生长活性，特别是对 EGFR 中度和低度表达的肿瘤细胞也显示出明显的细胞生长抑制活性，且细胞毒性低，因而具有良好的应用前景。

以下对本发明做进一步描述:

在本发明中，除非另有说明，否则本文中使用的科学和技术名词具有本领域技术人员所通常理解的含义。并且，本文中所用的蛋白质和核酸化学、分子生物学、细胞和组织培养、微生物学、免疫学相关术语和实验室操作步骤均为相应领域内广泛使用的术语和常规步骤。同时，为了更好地理解本发明，下面提供相关术语的定义和解释。

在本发明中，术语“抗体”是指通常由两对相同的多肽链（每对具有一条“轻”（L）链和一条“重”（H）链）组成的免疫球蛋白分子。抗体的轻链可分为 κ 和 λ 两类。重链可分为 μ、δ、γ、α 或 ε 五种，依据重链的不同可将抗体分为 IgM、IgD、IgG、IgA 和 IgE 五类。在轻链和重链内，可变区和恒定区通过大约 12 或更多个氨基酸的“J”区连接，重链还包含大约 3 个或更多个氨基酸的“D”区。各重链由重链可变区(V_H)和重链恒定区(C_H)组成。重链恒定区由 3 个结构域(C_H1、C_H2 和 C_H3)组成。各轻链由轻链可变区(V_L)和轻链恒定区(C_L)组成。轻链恒定区由一个结构域 C_L 组成。抗体的恒定区可介导免疫球蛋白与宿主组织或因子，包括免疫系统的各种细胞(例如，效应细胞)和补体系统的组分 C1q 的结合。V_H 和 V_L 区还可被细分为具有高变性的区域(称为互补决定区(CDR))，其间散布有较保守的称为骨架区（FR）的区域。各 V_H 和 V_L 由按下列顺序：FR1、CDR1、FR2、CDR2、FR3、CDR3、FR4 从氨基末端至羧基末端排列的 3 个 CDR 和 4 个 FR 组成。各重链/轻链对的可变区(V_H 和 V_L)分别形成抗体结合部位。氨基酸至各区域或结构域的分配遵循 Kabat Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and 1991))，或 Chothia & Lesk (1987) J. Mol. Biol. 196:901-917; Chothia 等人 (1989) Nature 342:878-883 的定义。术语“抗体”不受任何特定的

产生抗体的方法限制。例如，其包括，特别地，重组抗体、单克隆抗体和多克隆抗体。抗体可以是不同类型的抗体，例如，IgG (例如，IgG1，IgG2，IgG3 或 IgG4 亚型)，IgA1，IgA2，IgD，IgE 或 IgM 抗体。

在本发明中，所述 IgG 重链恒定区包括 IgG1、 IgG2、IgG3 或 IgG4。在本发明的实施方案中，所述 IgG 重链恒定区为 IgG1 型。

在本发明中，所述 κ 轻链恒定区包括各种同种异型，如 Km1、Km1,2 或 Km3。

关于抗体的氨基酸序列

在本发明的实施方案中，抗表皮生长因子受体抗体的重链可变区氨基酸序列是 SEQ ID NO:1。在本发明的实施方案中，抗表皮生长因子受体抗体的轻链可变区氨基酸序列是 SEQ ID NO:2。

另一方面，本发明所述的抗体的重链可变区的氨基酸序列与 SEQ ID NO:1 的序列相似性至少达 70％，优选是至少 75％，优选是至少 80％，优选是 85％，再优选是至少 90％，最好的是至少 95％。

另一方面，本发明所述的抗体的轻链可变区的氨基酸序列与 SEQ ID NO:2 的序列相似性至少达 70％，优选是至少 75％，优选是至少 80％，优选是 85％，再优选是至少 90％，最好的是至少 95％。

在本发明的实施方案中，抗表皮生长因子受体抗体的重链和轻链可变区的 CDR 的氨基酸序列确定如下：

重链的 CDR1、CDR2 和 CDR3 的氨基酸序列分别为 SEQ ID NO: 5-7；轻链的 CDR1、CDR2 和 CDR3 的氨基酸序列分别 SEQ ID NO: 12-14。

另一方面，抗表皮生长因子受体抗体的重链的 CDR 含有的氨基酸序列可能是在 SEQ ID NO: 5-7 上出现一个或多个氨基酸的突变或增添或缺失。优选的是，突变、添加或缺失的氨基酸不超过 3 个氨基酸。更优选的是，突变、添加或缺失的氨基酸不超过 2 个氨基酸。最优选的是，突变、添加或缺失的氨基酸不超过 1 个氨基酸。

另一方面，抗表皮生长因子受体抗体的轻链的 CDR 含有的氨基酸序列可能是在 SEQ ID NO: 12-14 上出现一个或多个氨基酸的突变、增添或缺失。优选的是，突变、添加或缺失的氨基酸不超过 3 个氨基酸。

更优选的是，突变、添加或缺失的氨基酸不超过 2 个氨基酸。最优选的是，突变、添加或缺失的氨基酸不超过 1 个氨基酸。

在本发明的实施方案中，抗表皮生长因子受体抗体的重链和轻链可变区的 FR 的氨基酸序列确定如下：

重链可变区 FR1、FR2、FR3、FR4 的序列分别为 SEQ ID NO: 8-11。轻链可变区 FR1、FR2、FR3、FR4 的序列分别为 SEQ ID NO: 15-18。

另一方面，抗表皮生长因子受体抗体的重链或轻链可变区 FR 的氨基酸序列可能是在 SEQ ID NO: 8-11、SEQ ID NO: 15-18 上出现一个或多个氨基酸的突变、增添或缺失。优选的是，突变、添加或缺失的氨基酸不超过 3 个氨基酸。更优选的是，突变、添加或缺失的氨基酸不超过 2 个氨基酸。最优选的是，突变、添加或缺失的氨基酸不超过 1 个氨基酸。

上述抗体或 CDR 区或框架区的氨基酸发生突变、添加或缺失之后的变异体仍然保留特异性结合 EGFR 的能力。

在本发明的实施方案中，抗表皮生长因子受体抗体重链恒定区氨基酸序列是 SEQ ID NO:3。在本发明的实施方案中，抗表皮生长因子受体抗体的轻链恒定区氨基酸序列是 SEQ ID NO:4。

另一方面，本发明所述的抗体的重链恒定区的氨基酸序列与 SEQ ID NO:3 的序列相似性至少达 70％，优选是至少 75％，优选是至少 80％，优选是 85％，再优选是至少 90％，最好的是至少 95％，如 96%、97%、98%、99%。

另一方面，本发明所述的抗体的轻链恒定区的氨基酸序列与 SEQ ID NO:4 的序列相似性至少达 70％，优选是至少 75％，优选是至少 80％，优选是 85％，再优选是至少 90％，最好的是至少 95％，如 96%、97%、98%、99%。

本发明所述的单抗变异体可以通过传统的基因工程方法获得。本领域的技术人员完全知晓利用核酸突变改造 DNA 分子的方法。另外，编码重链和轻链变异体的核酸分子也可以通过化学合成获得。

在本发明中，用于确定序列同一性（同源性）和序列相似性百分数的算法是例如 BLAST 和 BLAST 2.0 算法，它们分别描述在 Altschul 等（1977）Nucl. Acid. Res. 25: 3389-3402 和 Altschul 等（1990）J. Mol. Biol. 215: 403-410。采用例如文献中所述或者默认参数，BLAST 和 BLAST 2.0 可以用于确定本发明的氨基酸序列同一性百分数。执行 BLAST 分析的软件可以通过国立生物技术信息中心为公众所获得。

在本发明中，所述与氨基酸序列具有至少 70％的序列同一性的氨基酸序列包括与所述氨基酸序列基本同一的多肽序列，例如当采用本文所述方法（例如采用标准参数的 BLAST 分析）时，与本发明多肽序列相比含有至少 70％序列同一性、优选至少 75％、80％、85％、86％、87％、88％、89％、90％、91%、92%、93%、94%、95%、96%、97%、98%、99％或更高的序列同一性的那些序列。

在本发明中，用于抗体药物偶联物的毒素包括白喉毒素 A 链、白喉毒素的非结合活性片段、外毒素 A 链(来自铜绿假单胞菌(Pseudomonas aeruginosa))、蓖麻毒蛋白(ricin)A 链、相思豆毒蛋白(abrin)A 链、蒴莲根毒蛋白(modeccin)A 链、α-帚曲霉素(sarcin)、油桐(Aleutites fordii)毒蛋白、香石竹(dianthin)毒蛋白、美洲商陆(Phytolacaamericana)毒蛋白(PAPI、PAPII 和 PAP-S)、苦瓜(Momordica charantia)抑制物、麻疯树毒蛋白(curcin)、巴豆毒蛋白(crotin)、肥皂草(sapaonaria officinalis)抑制物、白树毒蛋白(gelonin)、丝林霉素(mitogellin)、局限曲菌素(restrictocin)、酚霉素(phenomycin)、依诺霉素(enomycin)、和单端孢菌素(trichothecenes)。参见例如 1993 年 10 月 28 日公布的 WO 93/21232。

多种放射性核素可用于生成抗体药物偶联物。例子包括 ^{212}Bi、^{131}I、^{131}In、^{90}Y、和 ^{186}Re。

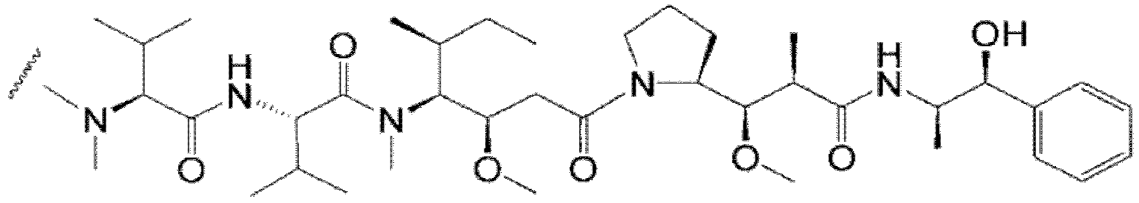
抗体和细胞毒剂的偶联物可使用多种双功能蛋白质偶联剂来制备，诸如 N-琥珀酰亚氨基 3-(2-吡啶基二硫代)丙酸酯(SPDP)，亚氨基硫烷(IT)，亚氨酸酯(诸如盐酸己二酰亚氨酸二甲酯)、活性酯类(诸如辛二酸

二琥珀酰亚氨基酯)、醛类(诸如戊二醛)、双叠氮化合物(诸如双(对-叠氮苯甲酰基)己二胺)、双重氮衍生物(诸如双(对-重氮苯甲酰基)-乙二胺)、二异硫氰酸酯(诸如甲苯 2，6-二异氰酸酯)、和双活性氟化合物(诸如 1，5-二氟-2，4-二硝基苯)的双功能衍生物。例如，可如 Vitetta 等在(1987)Science，238: 1098 中所述制备的含蓖麻毒的免疫毒素。碳-14 标记的 1-异硫氰酸苄基-3-甲基二亚乙基三胺五乙酸(MX-DTPA)是用于将放射性核苷酸与抗体偶联的例示性螯合剂(WO94/11026)。

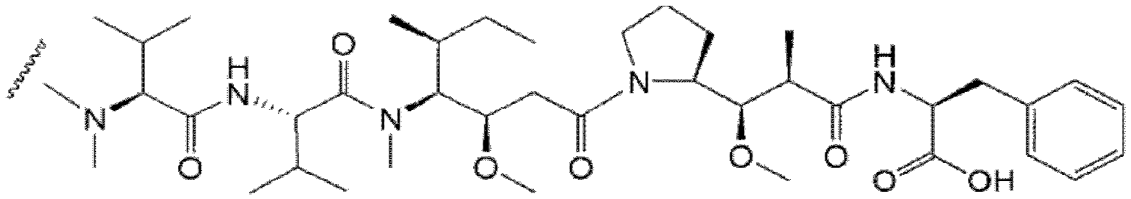
本发明还涵盖抗体和一种或多种小分子毒素(诸如加利车霉素(calicheamicin)、美登素生物碱(maytansinoid)、多拉司他汀(dolastatin)、auristatin、单端孢霉素(trichothecene)、和 CC 1065 及这些毒素的具有毒素活性的衍生物的偶联物。

在有些实施方案中，抗体药物偶联物包含与多拉司他汀(dolastatin)或多拉司他汀肽类似物和衍生物 auristatin(美国专利 No.5,635,483; 5,780,588)偶联的抗表皮生长因子受体抗体。多拉司他汀和 auristatin 已经显示出干扰微管动力学、GTP 水解、及核和细胞分裂等活性(Woyke 等(2001)Antimicrob.Agents and Chemother.45(12): 3580-3584)且具有抗癌 (US 5,663,149) 和抗真菌活性 (Pettit 等 (1998) Antimicrob.Agents Chemother.42: 2961-2965)。多拉司他汀或 auristatin 药物模块可经由肽药物模块的 N(氨基)末端或 C(羧基)末端附着于抗体(WO 02/088172)。

在本发明中，其中 MMAE 的结构为:



在本发明中，其中 MMAF 的结构为:



在本发明中，作为细胞毒剂的酶可以为具有核酸降解活性的化合物(例如核糖核酸酶或 DNA 内切核酸酶，诸如脱氧核糖核酸酶；DNA 酶)。

在本发明中，药物载荷(loading)由 p 表示，即式Ⅰ：$Ab-(L-D)_p$ 的分子中每个抗体的平均药物模块（即细胞毒剂）数。药物载荷的范围可以为每个抗体 1-20 个药物模块(D)。通式 I 的 ADC 包括偶联有一定范围(1-20 个)药物模块的抗体的集合。来自偶联反应的 ADC 制备物中每个抗体的平均药物模块数可以通过常规手段来验证，诸如质谱、ELISA 测定法、和 HPLC。还可以测定 ADC 在 p 方面的定量分布。在有些情况中，将 p 为某数值的同质 ADC 从具有其它药物载荷的 ADC 中分离、纯化和验证可以通过诸如反相 HPLC 或电泳的手段来实现。

对于有些抗体-药物偶联物，p 可能受到抗体上附着位点数目的限制。例如，若附着是半胱氨酸硫醇，抗体可能只有一个或数个半胱氨酸硫醇基，或者可能只有一个或数个有足够反应性的硫醇基，可附着接头。在某些实施方案中，较高的药物载荷，例如 $p>5$，可引起某些抗体-药物偶联物的聚集、不溶性、毒性、或丧失细胞通透性。

在某些实施方案中，本发明 ADC 的药物载荷范围为 1 到约 8；约 2 到约 6；约 3 到约 5；约 4 到约 5；约 3.5 到 4.5。事实上，对于某些 ADC 已经显示了每个抗体的药物模块的最佳比率可以为小于 8，可以为约 2 到约 5。参见 US2005-0238649A1(完整收入本文作为参考)。

在某些实施方案中，在偶联反应中少于理论最大值的药物模块偶联至抗体。抗体可包含例如赖氨酸残基，其不与药物-接头中间物或接头试剂起反应。一般而言，抗体不包含许多游离的和反应性的半胱氨酸硫醇基，其可连接药物模块；事实上，抗体中的大多数半胱氨酸硫醇基以二硫桥形式存在。在某些实施方案中，可以在部分或完全还原性条件下用还原剂诸如二硫苏糖醇(DTT)或三羰基乙基膦(TCEP)还原抗体以产生反应性半胱氨酸硫醇基。在某些实施方案中，将抗体置于变性条件以暴

露反应性亲核基团，诸如赖氨酸或半胱氨酸。

ADC 的载荷(药物/抗体比率)可以以不同方式来控制，例如通过：(i)限制药物-接头中间物或接头试剂相对于抗体的摩尔数，(ii)限制偶联反应的时间或温度，(iii)半胱氨酸硫醇修饰的部分或限制还原性条件，(iv)通过重组技术对抗体的氨基酸序列进行工程改造，使得半胱氨酸残基的数目和位置为了控制接头-药物附着的数目和/或位置而进行改变。应当理解，若超过一个亲核基团与药物-接头中间物或者与接头试剂和接下来的药物模块试剂起反应，则所得产物是具有一个或多个药物模块附着于抗体的 ADC 化合物混合物。可以通过对抗体特异性和对药物特异性的双重 ELISA 抗体测定法自混合物计算每个抗体的平均药物数。混合物中的各种 ADC 分子可以通过质谱来鉴定，并通过 HPLC 来分离，例如疏水相互作用层析。在某些实施方案中，可以通过电泳或层析从偶联混合物中分离具有单一载荷值的同质 ADC。

在本发明中，抗体药物偶联物的药学上可接受的盐包括无机酸、羧酸和磺酸的酸加成盐，例如以下酸的盐：盐酸、氢溴酸、硫酸、磷酸、甲烷磺酸、乙烷磺酸、苯磺酸、甲苯磺酸、萘二磺酸、乙酸、三氟乙酸、丙酸、乳酸、酒石酸、苹果酸柠檬酸、富马酸、马来酸和苯甲酸。

本发明的抗体药物偶联物的药学上可接受的盐还包括常规碱的盐，例如(仅为示例性并优选) 碱金属盐(例如钠盐和钾盐)、碱土金属盐(例如钙盐和镁盐) 和衍生自氨或含有1-16个碳原子的有机胺的铵盐，所述有机胺例如(仅为示例性并优选) 乙胺、二乙胺、三乙胺、乙基二异丙胺、单乙醇胺、二乙醇胺、三乙醇胺、二环己胺、二甲基氨基乙醇、普鲁卡因、二苯甲酰胺、N- 甲基哌啶、N- 甲基吗啉、精氨酸、赖氨酸和1，2-乙二胺。

在本发明中，溶剂合物表示这些形式的本发明的抗体药物偶联物：所述抗体药物偶联物通过与溶剂分子配位而形成的固态或液态形式的复合物。水合物是溶剂合物的一种具体形式，其具有配位的水分子。在本发明中，水合物是优选的溶剂合物。

可以优选地使用本发明的抗体药物偶联物进行治疗的与EFGR相关

的肿瘤包括过表达EGFR 的肿瘤、呼吸道肿瘤(例如小细胞癌和非小细胞癌、支气管癌)，其中特别优选非小细胞肺癌；消化道(例如食道、胃、胆囊、小肠、大肠、直肠) 的肿瘤，其中特别优选肠肿瘤；内分泌腺和外分泌腺(例如甲状腺和甲状旁腺、胰腺和唾液腺) 的肿瘤，其中特别优选胰腺肿瘤；头颈区域(例如喉、下咽、鼻咽、口咽、唇、口腔、舌和食道) 的肿瘤；和/ 或神经胶质瘤。

本发明的抗体药物偶联物可以与已知的用于治疗肿瘤的化疗药物联用，所述化疗药物例如为阿霉素(Adriamycin)、环磷酰胺和紫杉烷类[紫杉醇(Taxol)和多西他赛(Taxotere)]、卡培他滨(Xeloda)、吉西他滨(Gemzar)、长春瑞滨(Navelbine)、他莫昔芬、芳香酶抑制剂(瑞宁得、弗隆、阿诺新)、5-FU 加亚叶酸、伊立替康(camptosar)、奥沙利铂、顺铂、卡铂、雌莫司汀、米托蒽醌(Novantrone)、泼尼松、长春新碱(Oncovin)等，或它们的组合。

在本发明中，“治疗”指试图改变所治疗个体或细胞的自然进程的临床干预，可以是为了预防或在临床病理学的进程中进行。治疗的期望效果包括预防疾病的发生或复发、缓解症状、削弱疾病的任何直接或间接病理学后果、预防转移、减缓疾病进展的速率、改善或减轻疾病状态、及免除或改善预后。在有些实施方案中，本发明的抗体或抗体药物偶联物用于延迟疾病或病症的发生，或用于减缓疾病或病症的进展。用于评估疾病的成功治疗和改善的上述参数可以容易的通过内科医师所熟悉的常规规程来测量。对于癌症治疗，可通过例如评估疾病进展前时间(TTP)和/或测定反应率(RR)来测量功效。

在本发明中，“受试者”指脊椎动物。在某些实施方案中，脊椎动物指哺乳动物。哺乳动物包括，但不限于，牲畜(诸如牛)、宠物(诸如猫、犬、和马)、灵长类动物、小鼠和大鼠。在某些实施方案中，哺乳动物指人。

在本发明中，“有效量”指在必需的剂量和时间上有效实现期望的治疗或预防效果的量。本发明的物质/分子的“治疗有效量”可根据诸如个体的疾病状态、年龄、性别和体重及该物质/分子在个体中引发期望应答的

能力等因素而变化。治疗有效量还涵盖该物质/分子的治疗有益效果胜过任何有毒或有害后果的量。“预防有效量”指在必需的剂量和时间上有效实现期望的预防效果的量。通常而非必然，由于预防剂量是在疾病发作之前或在疾病的早期用于受试者的，因此预防有效量会低于治疗有效量。在癌症的情况中，药物的治疗有效量可减少癌细胞数；缩小肿瘤体积；抑制(即一定程度的减缓，优选停止)癌细胞浸润到周围器官中；抑制(即一定程度的减缓，优选停止)肿瘤转移；一定程度的抑制肿瘤生长；和/或一定程度的减轻与癌症有关的一种或多种症状。

对于疾病的预防或治疗，本发明的抗体药物偶联物的适宜剂量(在单独地或联合一种或多种其它别的治疗剂诸如化疗剂地使用时)会取决于待治疗疾病的类型、抗体药物偶联物的类型、疾病的严重程度和进程、施用抗体药物偶联物是出于预防还是治疗目的、先前的疗法、患者的临床病史和对抗体药物偶联物的反应性、及主治医师的判断。合适的是，一次性或通过一系列治疗将抗体药物偶联物施用于患者。根据疾病的类型和严重程度，施用于患者的初始候选剂量可以是约 1μg/kg 至 100mg/kg(例如 0.1mg/kg-20mg/kg)抗体药物偶联物，例如或是通过一次或多次分开施药或是通过连续输注。根据上文所述因素，典型日剂量的范围可以是约 1μg/kg 至 100mg/kg 或更多。对于持续数天或更长时间的重复施药，根据状况，通常持续治疗直至疾病症状发生期望的抑制。抗体药物偶联物的例示剂量的范围可以是约 0.05mg/kg 至约 10mg/kg。如此，可以对患者施用一剂或多剂约 0.5mg/kg、2.0mg/kg、4.0mg/kg 或 10mg/kg(或其任意组合)的抗体药物偶联物。此类剂量可间歇施用，例如每周或每三周(例如使得患者接受约 2 剂至约 20 剂，或例如约 6 剂抗体药物偶联物)。可施用一剂较高的初始加载剂量，后续一剂或多剂较低剂量。这种疗法的进程易于通过常规技术和测定法来监测。“长期”施用指与短期模式相反，以连续模式施用药剂，从而将初始治疗效果(活性)维持较长一段时间。“间歇”施用指不是无间断连续进行的治疗，而是本质上周期性的。“联合”一种或多种其它治疗剂的施用包括同时(共同)施用和任意次序的序贯施用。

“药学上可接受的载体”在用于本发明时包括药学可接受的载体、赋形剂或稳定剂，它们在所采用的剂量和浓度对暴露于其的细胞或哺乳动物是无毒的。通常，生理学可接受的载体是 pH 缓冲水溶液。生理学可接受载体的例子包括缓冲剂，诸如磷酸盐、柠檬酸盐和其它有机酸；抗氧化剂，包括抗坏血酸；低分子量(少于约 10 个残基)多肽；蛋白质，诸如血清清蛋白、明胶或免疫球蛋白；亲水性聚合物，诸如聚乙烯吡咯烷酮；氨基酸，诸如甘氨酸、谷氨酰胺、天冬酰胺、精氨酸或赖氨酸；单糖、二糖和其它碳水化合物，包括葡萄糖、甘露糖，蔗糖，海藻糖或糊精；螯合剂，诸如 EDTA；糖醇，诸如甘露醇或山梨醇；成盐反荷离子，诸如钠；和/或非离子表面活性剂，诸如 TWEEN™、聚乙二醇(PEG)和 PLURONICS™。

在本发明中，20 种常规氨基酸和其缩写遵从常规用法。参见 Immunology-A Synthesis (第 2 版, E.S. Golub 和 D.R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991))，其通过引用合并入本文。

附图说明

图 1 测定抗体药物偶联物的药物/抗体比例的 HIC-HPLC 图。

图 2 单抗及抗体药物偶联物的体外细胞活性测定，其中○代表 JMT101 单克隆抗体，▲代表 MRG003 抗体药物偶联物。

图 3 MRG003 对结肠癌细胞 HT-29 的生长抑制活性，其中○代表 JMT101 单克隆抗体，▲代表 MRG003 抗体药物偶联物。

图 4 MRG003 对脑胶质瘤癌细胞 U87-MG 的生长抑制活性，其中○代表 JMT101 单克隆抗体，▲代表 MRG003 抗体药物偶联物。

图 5 MRG003 对肺癌细胞 A549 的生长抑制活性，其中○代表 JMT101 单克隆抗体，▲代表 MRG003 抗体药物偶联物。

图 6 MRG003 对 KRAS 突变体结肠癌细胞 LoVo 的生长抑制活性，其中◇代表爱必妥单克隆抗体，▲代表 MRG003 抗体药物偶联物。

图 7 单抗及抗体药物偶联物对小鼠 HT-29 结肠癌移植瘤体积的影响。图中数据用平均值±标准差表示；与缓冲液对照组相比，*表示 P＜0.05，**表示 P＜0.01，***表示 P＜0.001。

图 8 单抗及抗体药物偶联物对小鼠 HT-29 结肠癌移植瘤模型的体重的影响。

具体实施方式

下面将结合实施例对本发明的实施方案进行详细描述，但是本领域技术人员将会理解，下列实施例仅用于说明本发明，而不应视为限定本发明的范围。实施例中未注明具体条件者，按照常规条件或制造商建议的条件进行。所用试剂或仪器未注明生产厂商者，均为可以通过市购获得的常规产品。

本发明中的抗体 JMT-101 即中国发明专利申请 CN 103772504A 中的 BA03，其制备方法参考该专利申请中的实施例 3，该抗体各部分的序列如下：

重链可变区序列为：

QVQLQESGPGLVKPSETLSLTCTVSGFSLSNYDVHWVRQAPGKGLEWLGVIWSGGNTDYNTPFTSRLTISVDTSKNQFSLKLSSVTAADTAVYYCARALDYYDYEFAYWGQGTLVTVSS（SEQ ID NO: 1）。

其中下划线部分分别为 CDR1（SEQ ID NO: 5）、CDR2（SEQ ID NO: 6）、CDR3（SEQ ID NO: 7）；

未下划线部分分别为 FR1（SEQ ID NO: 8）、FR2（SEQ ID NO: 9）、FR3（SEQ ID NO: 10）、FR4（SEQ ID NO: 11）。

轻链可变区序列为：

EIVLTQSPDFQSVTPKEKVTITCRASQSIGTNIHWYQQKPDQSPKLLIKYASESISGIPSRFSGSGSGTDFTLTINSLEAEDAATYYCQQNNEWPTSFGQGTKLEIK（SEQ ID NO: 2）。

其中下划线部分分别为 CDR1（SEQ ID NO: 12）、CDR2（SEQ ID NO: 13）、CDR3（SEQ ID NO: 14）；

未下划线部分分别为 FR1（SEQ ID NO: 15）、FR2（SEQ ID NO: 16）、FR3（SEQ ID NO: 17）、FR4（SEQ ID NO: 18）。。

重链恒定区序列为:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK（SEQ ID NO: 3）。

轻链恒定区序列为:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC（SEQ ID NO: 4）。

实施例 1 抗体药物偶联物的制备方法

取 10 毫克的 JMT-101 抗体，使用 15mL 的 30KD 超滤装置置换到还原缓冲液 (25mM 硼酸钠, pH8.0, 25mM NaCl, 5 mM EDTA)中，共三次置换；终体积约为 1mL,转移至新的 Eppendorf 离心管（称重）中，并称重;检测蛋白浓度，计算蛋白总量。向抗体中加入 2.5 倍摩尔数的 DTT，室温保温 2 小时，连续混匀；使用 15ml 的 30KD 超滤装置置换到偶联缓冲液 （50mM Tris, pH7.2, 150mM NaCl, 5 mM EDTA）中，共三次置换。取浓缩液，以 A280 测定蛋白浓度，并称重，计算蛋白总量；取 10μl 样品，以 Ellman's 方法测定自由巯基数;

并以下列公式计算其自由巯基的摩尔浓度:

$$C_{thiol}=\frac{A412\times 112}{b\times 14150}\ (M)$$

b: 比色皿光路长度（通常 1cm）。

根据自由巯基的摩尔浓度和总蛋白溶液体积计算自由巯基摩尔数。

向还原后的抗体中加入 1.1 倍于自由巯基摩尔数的 vc-MMAE（购上海皓元化学科技有限公司）（溶于 DMSO），混匀后室温反应 2 小时，间断混匀。向反应体系中加入 20 倍于所投入之 vc-MMAE 摩尔数的 N-乙酰半胱氨酸于反应液中，混匀，静置 5 分钟。使用 15ml 的 30KD 超滤装置置换到偶联物储存液（20mM 柠檬酸钠(Na-citrate), 0.3% NaCl, 5% 海藻糖（Trehalose）, 0.05% TWeen-80, pH6.0）中，共三次置换，即得到抗体药物偶联物 MRG003；样品于 4℃保存。

药物/抗体比例的测定:

将制备好的抗体药物偶联物 MRG003 经过 HIC-HPLC 分析 （Jun Ouyang, Drug-To-Antibody(DAR) Ratio and Drug Distributionby Hydrophobic Interaction Chromatography and Reverse Phase High Performance Chromatography, Laurent Ducry(ed.), Antibody Drug Conjugates, Chapter 17, Methods in Molecular Biology, Vol 1045, p275-283）以测定药物/抗体比例 （drug antibody ratio， DAR），见图 1，根据图谱峰面积计算得到平均载药数 DAR 为 4.1。

实施例 2 抗体药物偶联物 MRG003 体外细胞活性的测定

细胞活性检测方法:

1.1 复苏后的细胞株经过 3-4 代细胞传代之后，先倒去培养基，用 5ML DPBS 润洗一遍，再用 3mL 胰蛋白酶消化细胞，分别用培养基重悬，离心机离心，弃上清，再用培养基再次重悬，取出 0.5mL 用细胞计数仪计数。在 96 孔细胞培养板上进行铺板（DiFi 细胞以 10000 个细胞/孔 HT-29 细胞以 5000 个细胞/孔, A549 细胞以 2000 个细胞/孔, U87-MG 细胞以 3000 个细胞/孔， LoVo 细胞以 4000 个细胞/孔），培养 24 小时后，加入系列稀释浓度的单克隆抗体 JMT-101 以及抗体药物偶联物 MRG003 保温 72 小时，之后每孔加入 20ul CCK8 显色试剂 在波长 450-650nm 用酶标仪检测 OD450-650，并进行四参数拟合。

体外细胞活性实验结果:

以下各细胞株购自中国科学院上海细胞生物学研究所。

在 EGFR 高表达的 DiFi 细胞（人结直肠癌细胞）中的活性: MRG003 比单抗 JMT-101 有显著增高的细胞生长抑制活性，EC50 降低了大约 10 倍（JMT-101 的 EC50 是 51.9 ng/ml， MRG003 的 EC50 是 5.1 ng/ml），如图 2 所示。

在 EGFR 中度表达和低度表达的其它肿瘤细胞中的活性: MRG003 相对于单抗本身对 EGFR 中度表达和低度表达的癌细胞（人结肠癌细胞 HT29、人肺癌细胞 A549、人脑星形胶质母细胞瘤细胞 U87-MG）也显示了明显的细胞生长抑制活性 （如图 3、图 4、图 5 所示），其中 HT-29 的 EC50 是 611 ng/ml, A549 的 EC50 是 28.3 μg/ml, U87-MG 的 EC50 是 5.3 μg/ml。

另外，我们还测试了在 KRAS 突变体结肠癌细胞 LoVo (Dunn EF, Ilda M, Myers RA, Hintz KA, Campbell DA, Armstrong EA, Li C and Wheeler DL. Dasatinib sensitizes KRAS mutant colorectal tumors to cetruximab. Oncogene 2011; 30:561-574)中的活性，发现 MRG003 对 KRAS 突变体结肠癌细胞 LoVo 显示了明显的肿瘤生长抑制活性（如图 6 所示，EC50 是 3.2μg/ml）。

实施例 3 小鼠体内移植瘤实验

小鼠体内移植瘤实验方法:

HT-29 结肠癌细胞是 EGFR 低表达的细胞株，目前已在市场上销售的治疗结直肠癌的 EGFR 靶向单抗爱必妥(Erbitux) 对 HT-29 细胞株没有生长抑制活性。

HT-29 细胞移植模型：收集对数生长期的肿瘤细胞，计数后重悬于 1×PBS，调整细胞悬液浓度至 3×10^7/ml。用 1ml 注射器（4 号针头）在裸鼠右侧背部皮下接种肿瘤细胞，3×10^6/0.1ml/鼠，当肿瘤体积达到 150-200mm^3 后用随机区组法分组，每组 8 只小鼠，保证各组间肿瘤体积和小鼠体重均一。各组肿瘤体积的均值与所有实验动物肿瘤体积的均

值差异不超过±10％。 尾静脉给药，每四天给一次药（第 1、5、9、13 天），一共给药 4 次，定期测量肿瘤体积和小鼠体重。每一个给药组分别有 8 只小鼠。

小鼠移植瘤实验结果

小鼠 HT-29 结肠癌移植瘤实验：实验共分五组， 包括缓冲液组（20 mM 柠檬酸钠，0.3%氯化钠，5%海藻糖，0.05%吐温 80， pH6），JMT-101 单抗组（5mg/kg）、MRG003 组（1mg/kg）、MRG003 组（5mg/kg）和非结合 ADC 组（5mg/kg）（人源 IgG-vcMMAE 偶联物，其中 IgG 是从健康人血清纯化的 IgG，该偶联物制备方法与 MRG003 相同）。MRG003 给药的小鼠移植肿瘤体积与对照组比较显著降低，显示了明显的抗肿瘤生长效应（图 7）。在第 18 天，MRG003 在 5mg/Kg 的给药剂量组与缓冲液组相比肿瘤生长抑制率达到 54%，与单抗 JMT-101 的同等剂量组相比肿瘤生长抑制率达到 46%， 与非结合 ADC 相比肿瘤生长抑制率达到 42%.

小鼠体重：MRG003 给药的小鼠体重与对照组比较没有变化（见图 8），说明 MRG003 没有使小鼠体重降低的毒性作用

尽管本发明的具体实施方式已经得到详细的描述，本领域技术人员将会理解。根据已经公开的所有教导，可以对那些细节进行各种修改和替换，这些改变均在本发明的保护范围之内。本发明的全部范围由所附权利要求及其任何等同物给出。

2015100850388

说　　明　　书　　附　　图

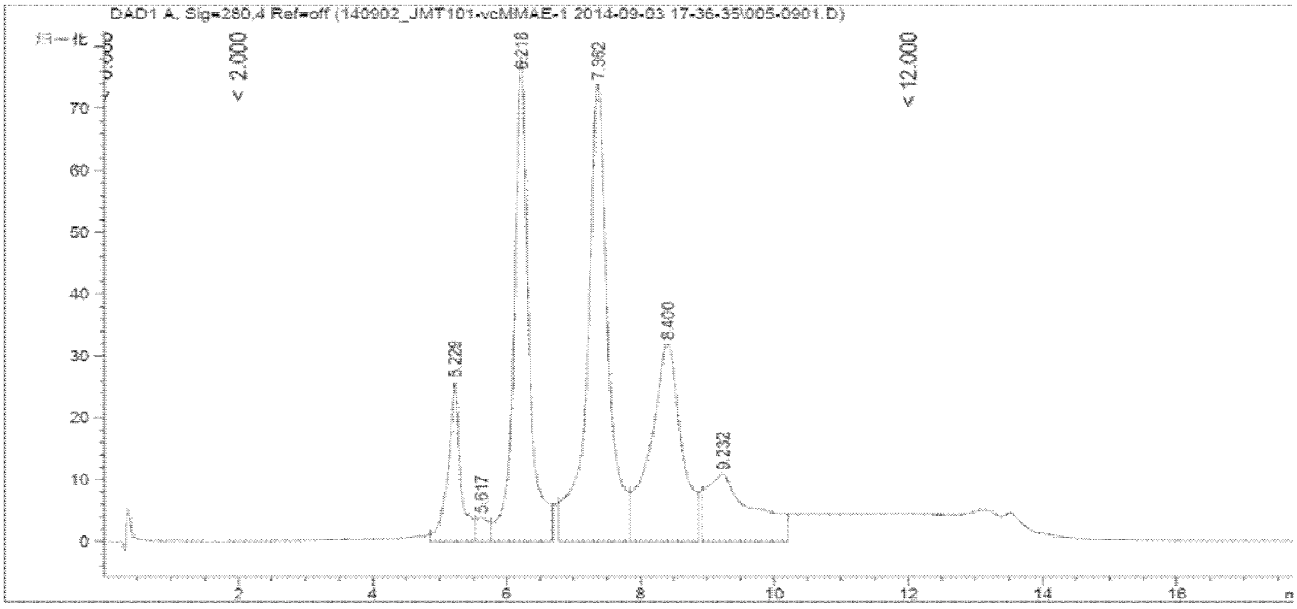


图1

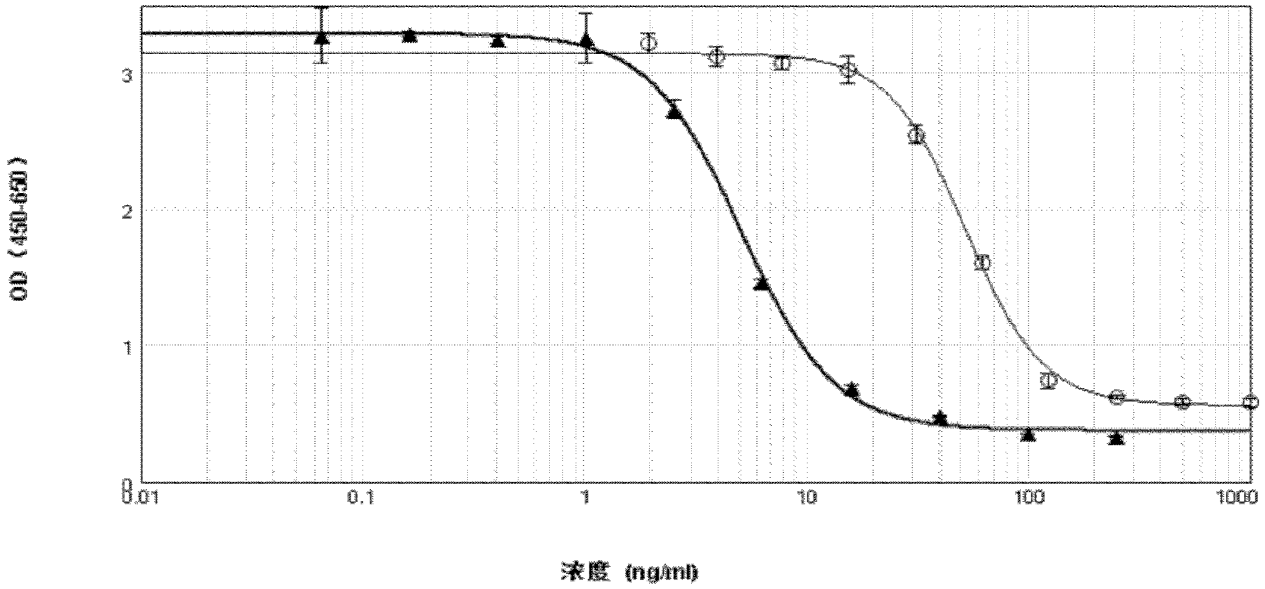


图2

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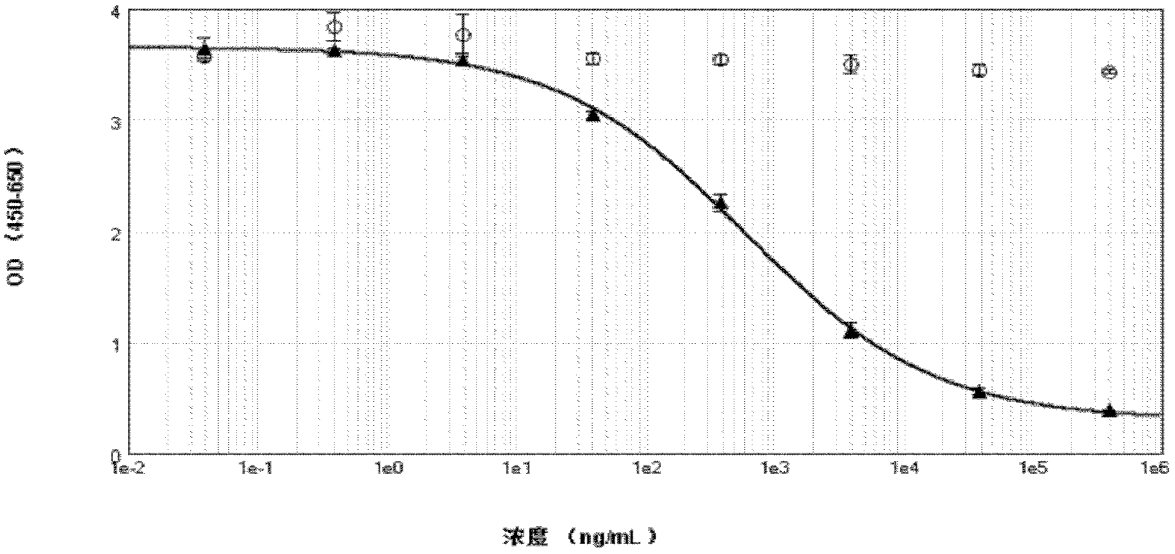


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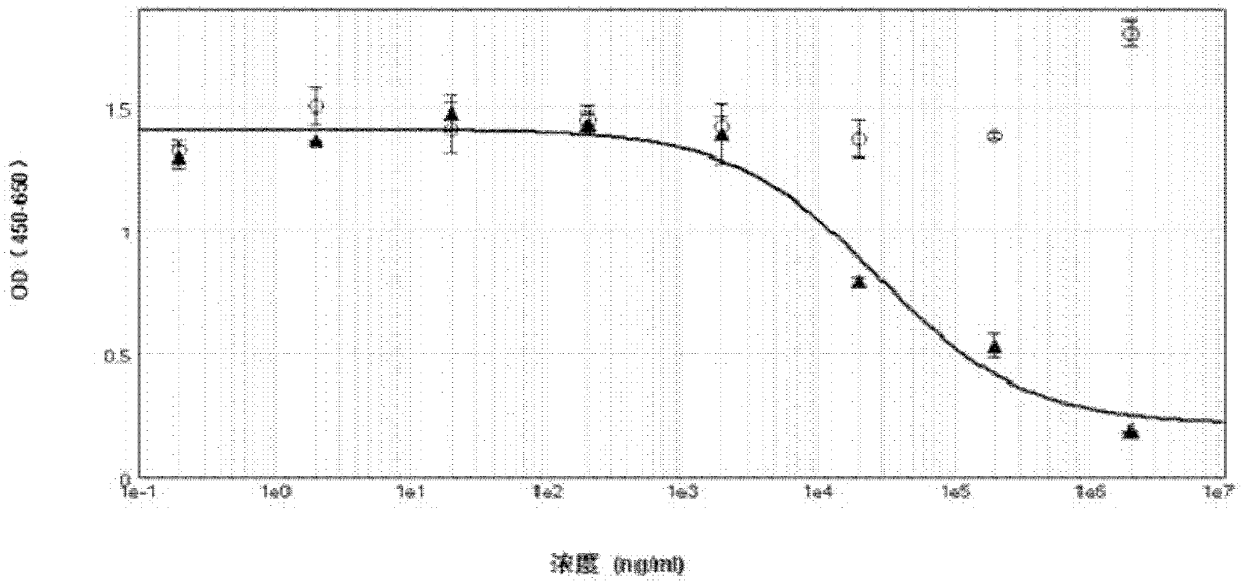


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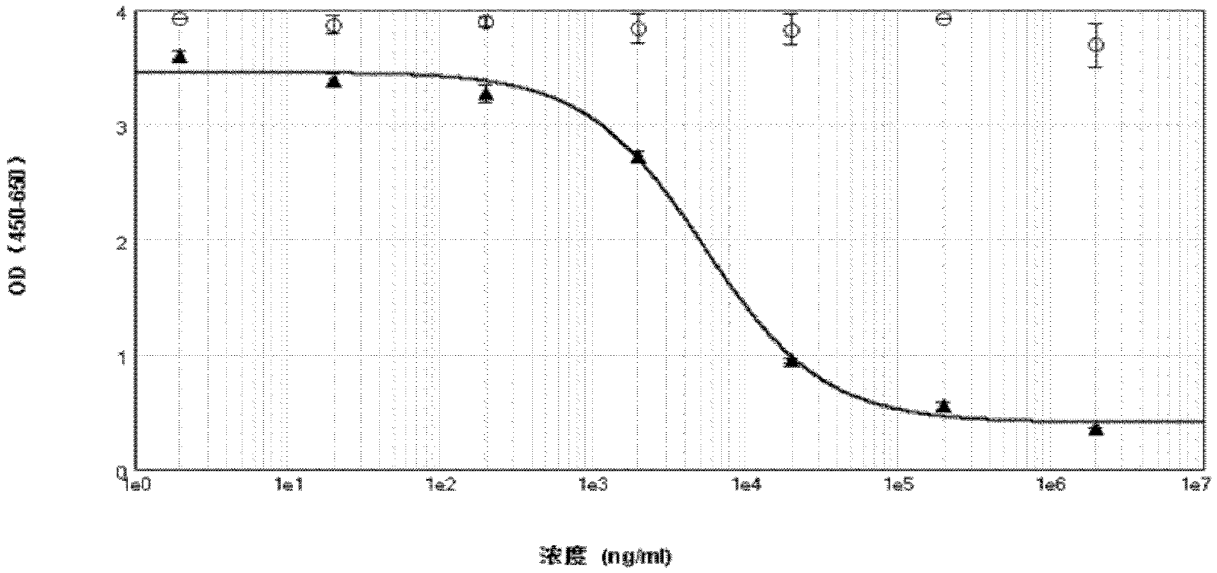

OD（450-650）
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1
2
3
4
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1e1
1e2
1e3
1e4
1e5
1e6
1e7
浓度（ng/ml）

图5

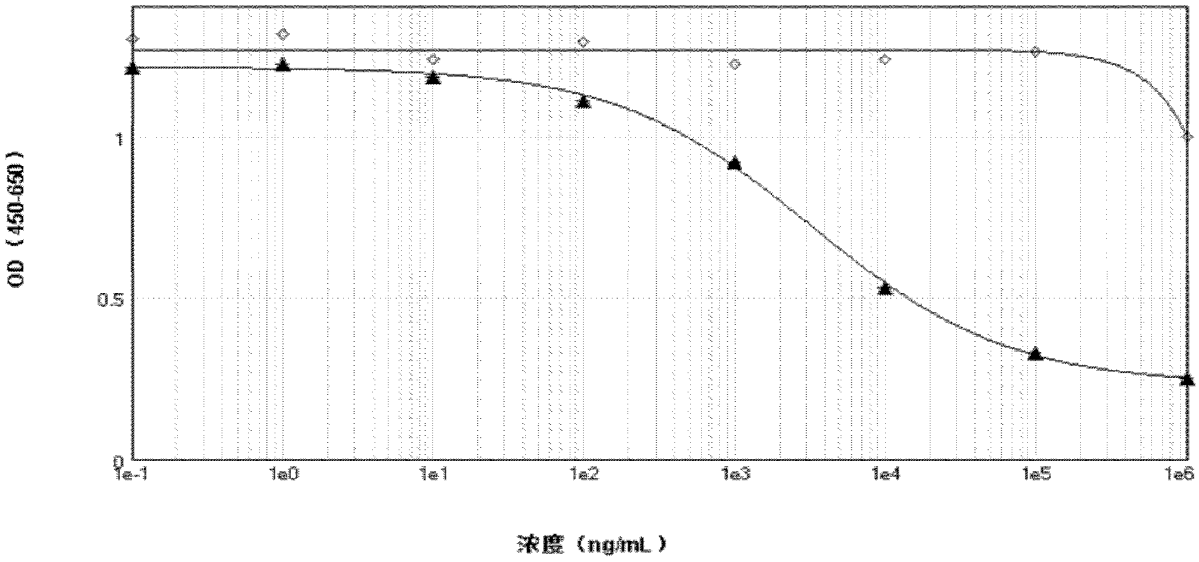

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浓度（ng/mL）

图6

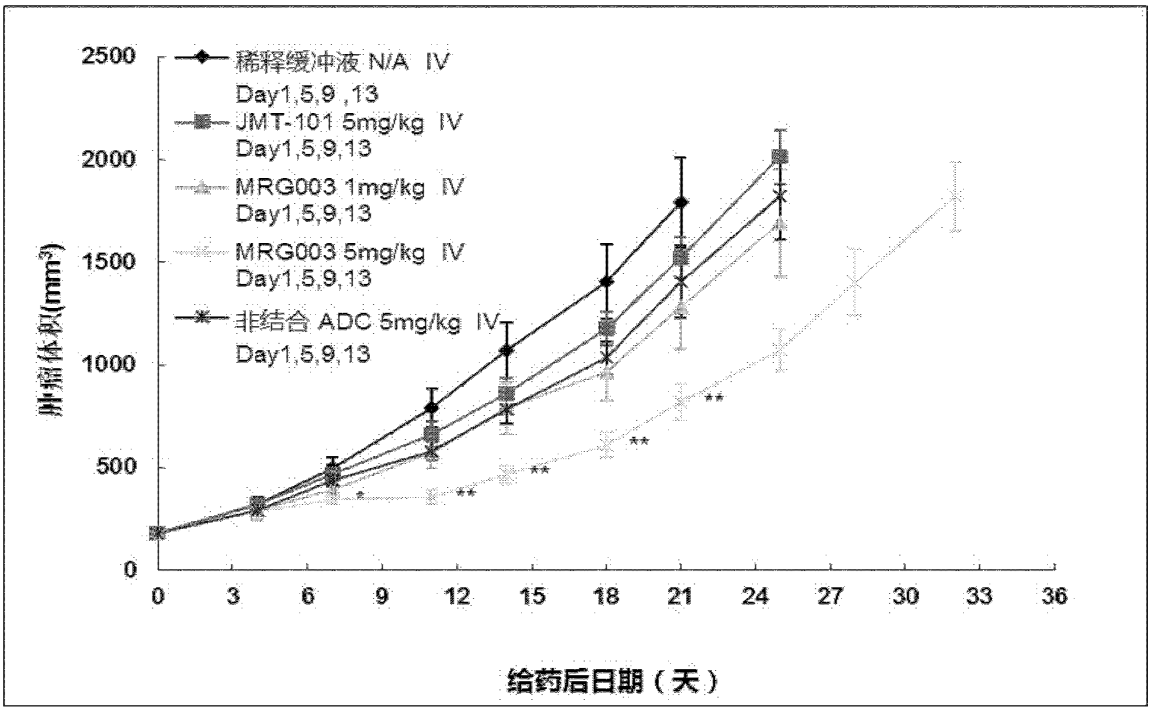

稀释缓冲液 N/A IV Day1,5,9 ,13
JMT-101 5mg/kg IV Day1,5,9,13
MRG003 1mg/kg IV Day1,5,9,13
MRG003 5mg/kg IV Day1,5,9,13
非结合 ADC 5mg/kg IV Day1,5,9,13
肿瘤体积(mm³)
给药后日期（天）

图7

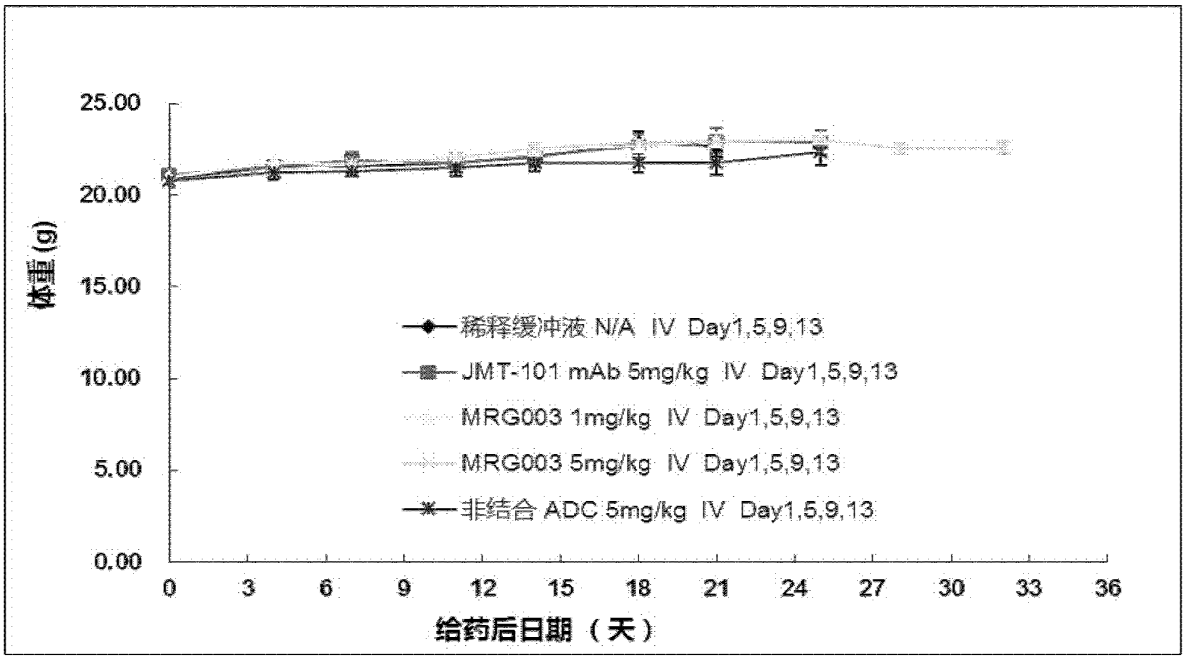

稀释缓冲液 N/A IV Day1,5,9,13
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MRG003 1mg/kg IV Day1,5,9,13
MRG003 5mg/kg IV Day1,5,9,13
非结合 ADC 5mg/kg IV Day1,5,9,13
体重 (g)
给药后日期（天）

图8

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Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
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(81) 指定国 (除另有指明，要求每一种可提供的国家保护): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW。

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根据细则 4.17 的声明:
— 发明人资格(细则 4.17(iv))

本国际公布:
— 包括国际检索报告(条约第 21 条(3))。
— 包括说明书序列表部分(细则 5.2(a))。

WO 2016/131409 A1

(54) Title: ANTIBODY-DRUG CONJUGATE

(54) 发明名称 : 抗体药物偶联物

(57) Abstract: Provided is an antibody-drug conjugate targeting an epidermal growth factor receptor (EGFR), and also disclosed are a composition comprising the antibody-drug conjugate and a use of the antibody-drug conjugate in the preparation of a drug for preventing and/or treating diseases associated with the epidermal growth factor receptor.

(57) 摘要: 提供了一种抗表皮生长因子受体抗体药物偶联物，还公开了包含该抗体药物偶联物的组合物和该抗体药物偶联物在制备预防和/或治疗与表皮生长因子受体有关的疾病的药物中的应用。

抗体药物偶联物

技术领域

本发明涉及抗体药物偶联物，具体涉及抗表皮生长因子受体抗体药物偶联物。本发明还涉及包含该抗体药物偶联物的组合物，以及该抗体药物偶联物的医药用途。

背景技术

表皮生长因子受体 (Epidermal Growth Factor Receptor，EGFR，也称为 HER1， c-ErbB1) 是表皮生长因子家族的细胞表面受体，是由 1186 个氨基酸残基构成，分子量为 170kD 的一种跨膜糖蛋白 (Jorissen RN，Walker F，Pouliot N，et al.Epidermal growth factor receptor：mechanisms of activation and signaling.Exp Cell Res，2003；284 ：31-53)。EGFR 属于Ⅰ型酪氨酸激酶受体 ErbB 亚族 (ErbB 1-4)，具有酪氨酸激酶的活性。EGFR 稳定的表达于许多上皮组织，包括皮肤和毛囊。表皮生长因子受体异常表达或因受体突变而活化会导致癌变。有很多实体瘤发现过度表达表皮生长因子受体，如结直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌和食管癌等 (Olayioye MA，Neve RM，Lane HA，et al.TheEerbB Signaling network ：receptor heterodimerzation in development and cancer.The EMBO J，2000 ；19 ：3159-3167)。转化生长因子 α 和表皮生长因子等生长因子是表皮生长因子受体的内源性配体。这些配体与表皮生长因子受体结合，激活受体胞内酪氨酸蛋白激酶的活性，启动下游多条信号转导途径，从而调节正常细胞的生长和分化，增加肿瘤细胞的侵袭力、促进血管生成、抑制肿瘤细胞的凋亡 (Ciardiello F，Tortora G.A novel approach in the treatment of cancer：targeting the epidermal growth factor receptor.Clin Cancer Res，2001；7 ：2958-2970)。表皮生长因子受体在肿瘤中的过表达及其在肿瘤细胞生长、分化中起重要作用的这些特点，使表皮生长因子受体成为具有良好前景的肿瘤治疗靶点。

目前市场上已有两个抗表皮生长因子受体的治疗性抗体，一个是人鼠嵌合抗体 C225 抗体 (爱必妥，Erbitux 或 Cetuximab，ImClone(现在 Eli Lilly) 公司)，与表皮生长因子受体有特异亲和性，可阻断 EGF 和 TGFα 等配体与表皮生长因子受体的结合，抑制其磷酸化和下游信号传导，从而抑制肿瘤细胞的生长，诱导凋亡，减少基质金属蛋白酶和血管上皮生长因子的

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产生。2004 年美国 FDA 批准爱必妥治疗结直肠癌，2006 年批准其治疗头颈部癌，目前有更多临床试验用于其它肿瘤适应症。在临床上，爱必妥和伊立替康合用治疗结直肠癌的有效率（ORR）为 23%，与氟嘧啶等化疗药物合用治疗头颈癌的有效率为 13%-30%。 由于是人鼠嵌合抗体，爱必妥在临床试验中有 3.7%的病人产生了抗体反应。

另一个抗表皮生长因子受体的治疗性抗体是帕尼单抗 (Vectibix, panitumumab，Amgen 公司)， 是采用转基因小鼠技术制备的全人化单克隆抗体，无鼠源蛋白序列。该抗体靶向作用于表皮生长因子受体 (EGFR)，2006 年 9 月被 FDA 批准上市，与氟嘧啶、奥沙利铂和伊立替康合用或在化疗后用于治疗 EGFR 阳性的转移性结直肠癌。2006 年 FDA 批准其单药治疗化疗耐受的转移性结直肠癌(mCRC)。然而帕尼单抗是 IgG2 亚型抗体，与 IgG1 相比，IgG2 的 CDC 活性及 ADCC 等生物学活性明显减低；另外，IgG2 亚型抗体稳定性较差，这可能是临床效果上全人抗体帕尼单抗与嵌合抗体爱必妥相比没有明显优势的主要原因。在临床上治疗结直肠癌的总体生存率（OR）才达到 8%，非进展生存期只延长了 3.6 个月。

目前大量的临床数据显示爱必妥单抗和帕尼单抗只对 EGFR 表达的 KRAS 野生型 （KRAS wild type）有疗效，而对 KRAS 突变体没有肿瘤生长抑制活性，因此， 美国临床肿瘤协会发表的指导原则中明确指出抗 EGFR 单抗药物只适用于 KRAS 野生型的结肠癌病人 （Allegra CJ, Jessup JM, Somerield MR, Hamilton SR, Hammond EH, Hayes DF, et al. American Soceitey of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor monoclonal antibody therapy. J. Clin Oncol. 2009; 27:2091-2096; Bardelli A, Siena S. Molecular mechanisms of resistance to cetuximab and panitumumab in colorectal cancer. J Clin Oncol. 2010; 28:1254-1261）。

因此，本领域亟需具有更高生物学活性的人源化抗表皮生长因子受体的抗体类药物，尤其是对 KRAS 突变体有疗效的抗体类药物，例如抗体药物偶联物，以进一步提高疗效，降低副作用。

由于单抗癌症药物的引领，近年来在欧美快速发展的抗体药物偶联物（antibody drug conjugate， ADC）成为目前最前沿的医药技术之一。

抗体药物偶联物一般由三个部分组成:

1. 专一性结合靶点的单抗;

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2. 具有细胞毒性的小分子化学药物;

3. 链接小分子药物和单抗的链接体，也叫接头。

抗体药物偶联物利用单抗的靶点专一性和化学药物的细胞毒性来杀死肿瘤细胞。它的作用机制是：（1）抗体药物偶联物利用单抗特异地结合到肿瘤细胞上的靶点抗原；（2）抗体药物偶联物和靶点抗原的复合物通过靶点抗原介导内吞进入细胞；（3）抗体药物偶联物在细胞内降解，释放具有细胞毒的化学药物；（4）具有细胞毒的化学药物杀死肿瘤细胞。

抗体药物偶联物的作用机制看似简单，但是一个抗体药物偶联物是否能成为安全有效的药物是非常复杂和不可预测的，依赖多种因素，例如：

1) 靶点的特性： 靶点是否能内吞噬 （internalize），靶点的表达水平，靶点是否在癌细胞和正常细胞中有足够的表达水平差异，靶点是否会脱落细胞外部分（extracellular domain, ECD）到血液中；

2) 单抗的特性： 单抗对靶点的特异性是否足够好 （与其它蛋白无交叉反应），单抗的稳定性如何，单抗和靶点的复合物是否能内吞噬入细胞；

3) 小分子化学药物的特性： 小分子化学药物的细胞毒性是否足够强，其在血液里的稳定性，以及其成为 ADC 后在体内的代谢产物的毒性；

4) 接头的特性： 接头是可切割的 （cleavable）还是不可切割的 (non-cleavable)，接头在血液里的稳定性；

5) ADC 的特性：ADC 和靶点的复合物是否能内吞噬，ADC 在血液里的稳定性，ADC 上用什么链接体和链接几个化学药物，ADC 杀死癌细胞的活性和生物毒性。

可以看出， ADC 药物的研发需要大量的实验摸索和验证，其安全性和有效性是无法在实验前预测的。

发明内容

本发明的发明人通过大量实验和创造性劳动，制备得到了抗表皮生长因子受体抗体药物偶联物，并证实其具有良好的生物学活性，由此完成了本发明。

本发明第一方面涉及抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其包含与细胞毒剂共价连接的抗表皮生长因子受体抗体。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体包括重链和轻链，其中重链可变区 CDR1、CDR2、CDR3 分别包含如 SEQ ID

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NO: 5～7所示的序列或其突变体，轻链可变区CDR1、CDR2、CDR3分别包含如SEQ ID NO: 12～14所示的序列或其突变体。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体轻链可变区CDR1、CDR2、CDR3区分别包含如SEQ ID NO: 12～14所示的序列或者与SEQ ID NO: 12～14所示的序列的同一性大于70%，例如大于75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%的序列，例如具有3个、2个或1个突变、缺失或添加氨基酸的序列。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体重链可变区CDR1、CDR2、CDR3区分别包含如SEQ ID NO: 5～7所示的序列或者与SEQ ID NO: 5～7所示的序列的同一性大于70%，例如大于75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%的序列，例如具有3个、2个或1个突变、缺失或添加氨基酸的序列。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体重链可变区FR1、FR2、FR3、FR4区分别包含如SEQ ID NO: 8～11所示的序列或其突变体。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体重链可变区FR1、FR2、FR3、FR4区分别包含如SEQ ID NO: 8～11所示的序列，或者包含与上述序列的同一性大于70%，例如大于75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%的序列。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体轻链可变区FR1、FR2、FR3、FR4区分别包含如SEQ ID NO: 15～18所示的序列或其突变体。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体轻链可变区FR1、FR2、FR3、FR4区分别包含如SEQ ID NO: 15～18所示的序列，或者包含与上述序列的同一性大于70%，例如大于75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%的序列。

在本发明的一个实施方案中，所述抗表皮生长因子受体抗体的重链可变区的序列如SEQ ID NO: 1所示。

在本发明的一个实施方案中，所述抗表皮生长因子受体抗体的轻链可

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变区的序列如 SEQ ID NO: 2 所示。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体的重链恒定区选自人源性 IgG、IgM、IgA、IgD、IgA 恒定区或上述恒定区的突变体。

在本发明的一个实施方案中，其中所述 IgG 选自 IgG1、IgG2、IgG3 和 IgG4。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体重链恒定区的氨基酸序列包含如 SEQ ID NO: 3 所示的序列，或者包含与 SEQ ID NO: 3 所示序列的同一性大于 70%，例如大于 75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%的序列。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体的轻链恒定区为人源性 lambda 恒定区、kappa 恒定区、或上述恒定区的突变体。

在本发明的一个实施方案中，其中所述的抗表皮生长因子受体抗体轻链恒定区的氨基酸序列包含如 SEQ ID NO: 4 所示的序列，或者包含与 SEQ ID NO: 4 所示序列的同一性大于 70%，例如大于 75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%的序列。

在本发明的一个实施方案中，其中所述的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物具有式 I 所示的结构，

$$Ab\text{-}(L\text{-}D)_p$$

式 I

其中：

Ab 代表抗表皮生长因子受体抗体；

L 代表接头；

D 代表细胞毒剂；

p 代表 1-8，例如 2-6，例如 3-5。

在本发明的一个实施方案中，其中所述的细胞毒剂选自化疗药物、毒素（例如细菌、真菌、植物、或动物源性的酶活性毒素或其片段）、放射性同位素、细胞因子、抗生素、酶、纳米颗粒和生物活性肽。

在本发明的一个实施方案中，其中所述的细胞毒剂选自 Monomethyl auristatin E（MMAE）、 Monomethyl auristatin F（MMAF）、 美登木素生物碱(例如Maytansine DM1、Maytansine DM4)、卡奇霉素(calicheamicin)、duocarmycin MGBA、阿霉素(doxorubicin)、蓖麻毒素、白喉毒素等毒素、

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I131、白介素类、肿瘤坏死因子、趋化因子和纳米颗粒等。

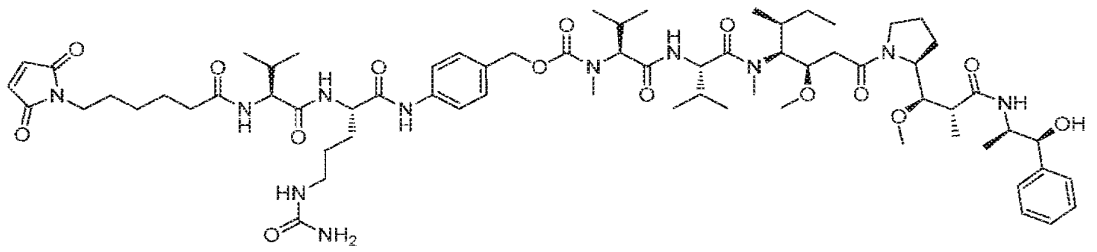
在本发明的一个实施方案中，其中所述的细胞毒剂为MMAE。

在本发明的一个实施方案中，其中所述的接头为可切割的或不可切割的。

在本发明的一个实施方案中，其中所述的接头选自 6-马来酰亚氨基己酰基(MC)、马来酰亚氨基丙酰基(MP)、缬氨酸-瓜氨酸(val-cit)、丙氨酸-苯丙氨酸(ala-phe)、对氨基苄氧羰基(PAB)、N-琥珀酰亚氨基 4-(2-吡啶基硫代)戊酸酯(SPP)、N-琥珀酰亚氨基 4-(N-马来酰亚氨基甲基)-环己烷-1-羧酸酯(SMCC)、N-琥珀酰亚氨基(4-碘-乙酰基)氨基苯甲酸酯(SIAB)、和 6-马来酰亚氨基己酰基-缬氨酸-瓜氨酸-对氨基苄氧羰基(MC-vc-PAB)。

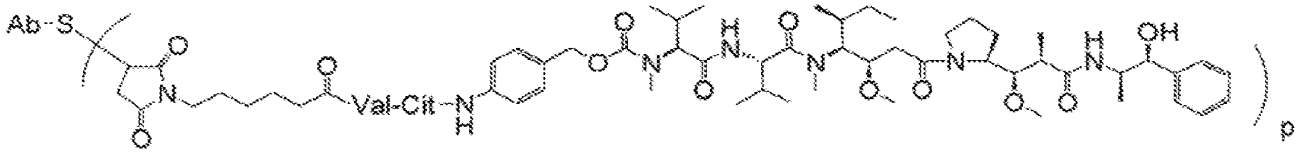
在本发明的一个实施方案中，其中所述的接头为 6-马来酰亚氨基己酰基-缬氨酸-瓜氨酸-对氨基苄氧羰基(MC-vc-PAB)。

在本发明的一个实施方案中，其中式 I 中所述的 L-D 为 vc-MMAE，其结构如下式所示：



。

在本发明的一个具体实施方案中，其中所述的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物如下式所示：



其中Ab为抗表皮生长因子受体抗体，p为1-8，例如2-6，例如3-5。

本发明第二方面涉及组合物（例如药物组合物），其含有本发明第一方面任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，任选地，还含有至少一种药学上可接受的载体、稀释剂或赋形剂。

在本发明的一个实施方案中，所述组合物中还包含已知的用于治疗肿瘤

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的化疗药物，所述化疗药物例如为阿霉素(Adriamycin)、环磷酰胺和紫杉烷类[紫杉醇(Taxol)和多西他赛(Taxotere)]、卡培他滨(Xeloda)、吉西他滨(Gemzar)、长春瑞滨(Navelbine)、他莫昔芬、芳香酶抑制剂(瑞宁得、弗隆、阿诺新)、5-FU 加亚叶酸、伊立替康(camptosar)、奥沙利铂、顺铂、卡铂、雌莫司汀、米托蒽醌(Novantrone)、泼尼松、长春新碱(Oncovin)等，或它们的组合。

本发明还涉及本发明第一方面任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物在制备预防和/或治疗与表面生长因子受体（EGFR）相关的疾病的药物中的用途。

在本发明的一个实施方案中，其中所述的与表面生长因子受体（EGFR）相关的疾病为与EGFR相关的肿瘤，例如与EGFR过表达相关的肿瘤，例如选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

在本发明一个实施方案中，其中所述肿瘤为KRAS基因突变的肿瘤，例如为KRAS基因突变的结肠癌、直肠癌、肺癌或胰腺癌。

在本发明一个实施方案中，其中所述的肿瘤为BRAF基因突变的肿瘤，例如选自BRAF基因突变的结肠癌、直肠癌和肺癌。

本发明还涉及本发明第一方面任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物在制备抑制肿瘤血管生成、延缓肿瘤进展、抑制肿瘤生长、抑制肿瘤细胞增殖的药物中的用途。

在本发明的一个实施方案中，其中所述的肿瘤选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

在本发明一个实施方案中，其中所述肿瘤为KRAS基因突变的肿瘤，例如为KRAS基因突变的结肠癌、直肠癌、肺癌或胰腺癌。

在本发明一个实施方案中，其中所述的肿瘤为BRAF基因突变的肿瘤，例如选自BRAF基因突变的结肠癌、直肠癌和肺癌。

本发明还涉及预防和/或治疗与表皮生长因子受体（EGFR）相关的疾病的方法，所述方法包括给予有需要的受试者预防和/或治疗有效量的本发明第一方面任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述

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盐的溶剂合物。

在本发明的一个实施方案中，其中所述的与表皮生长因子受体（EGFR）相关的疾病为与EGFR相关的肿瘤，例如与EGFR过表达相关的肿瘤，例如选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

在本发明一个实施方案中，其中所述肿瘤为KRAS基因突变的肿瘤，例如为KRAS基因突变的结肠癌、直肠癌、肺癌或胰腺癌。

在本发明一个实施方案中，其中所述的肿瘤为BRAF基因突变的肿瘤，例如选自BRAF基因突变的结肠癌、直肠癌和肺癌。

本发明还涉及抑制肿瘤血管生成、延缓肿瘤进展、抑制肿瘤生长、抑制肿瘤细胞增殖的方法，所述方法包括给予有需要的受试者预防和/或治疗有效量的本发明第一方面任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物。

在本发明的一个实施方案中，其中所述的肿瘤选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

在本发明一个实施方案中，其中所述肿瘤为KRAS基因突变的肿瘤，例如为KRAS基因突变的结肠癌、直肠癌、肺癌或胰腺癌。

在本发明一个实施方案中，其中所述的肿瘤为BRAF基因突变的肿瘤，例如选自BRAF基因突变的结肠癌、直肠癌和肺癌。

本发明还涉及本发明第一方面任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其用于预防和/或治疗与表皮生长因子受体（EGFR）相关的疾病。

在本发明的一个实施方案中，其中所述的与表皮生长因子受体（EGFR）相关的疾病为与EGFR相关的肿瘤，例如与EGFR过表达相关的肿瘤，例如选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

在本发明一个实施方案中，其中所述肿瘤为KRAS基因突变的肿瘤，例如为KRAS基因突变的结肠癌、直肠癌、肺癌或胰腺癌。

在本发明一个实施方案中，其中所述的肿瘤为BRAF基因突变的肿瘤，例如选自BRAF基因突变的结肠癌、直肠癌和肺癌。

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本发明还涉及本发明第一方面任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其用于抑制肿瘤血管生成、延缓肿瘤进展、抑制肿瘤生长、抑制肿瘤细胞增殖。

在本发明的一个实施方案中，其中所述的肿瘤选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

在本发明一个实施方案中，其中所述肿瘤为KRAS基因突变的肿瘤，例如为KRAS基因突变的结肠癌、直肠癌、肺癌或胰腺癌。

在本发明一个实施方案中，其中所述的肿瘤为BRAF基因突变的肿瘤，例如选自BRAF基因突变的结肠癌、直肠癌和肺癌。

本发明的抗表皮生长因子受体抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物在体内和体外都具有良好的抑制肿瘤细胞生长活性，特别是对 EGFR 中度和低度表达的肿瘤细胞也显示出明显的细胞生长抑制活性，且细胞毒性低；更特别地，本发明的抗表皮生长因子受体抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物对 KRAS 基因突变或 BRAF 基因突变的肿瘤也具有很好的疗效，因而具有良好的应用前景。

以下对本发明做进一步描述:

在本发明中，除非另有说明，否则本文中使用的科学和技术名词具有本领域技术人员所通常理解的含义。并且，本文中所用的蛋白质和核酸化学、分子生物学、细胞和组织培养、微生物学、免疫学相关术语和实验室操作步骤均为相应领域内广泛使用的术语和常规步骤。同时，为了更好地理解本发明，下面提供相关术语的定义和解释。

在本发明中，术语“抗体”是指通常由两对相同的多肽链（每对具有一条“轻”（L）链和一条“重”（H）链）组成的免疫球蛋白分子。抗体的轻链可分为 κ 和 λ 两类。重链可分为 μ、δ、γ、α 或 ε 五种，依据重链的不同可将抗体分为 IgM、IgD、IgG、IgA 和 IgE 五类。在轻链和重链内，可变区和恒定区通过大约 12 或更多个氨基酸的“J”区连接，重链还包含大约 3 个或更多个氨基酸的“D”区。各重链由重链可变区(V_H)和重链恒定区(C_H)组成。重链恒定区由 3 个结构域(C_H1、C_H2 和 C_H3)组成。各轻链由轻链可变区(V_L)和轻链恒定区(C_L)组成。轻链恒定区由一个结构域 C_L 组成。抗体的

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恒定区可介导免疫球蛋白与宿主组织或因子，包括免疫系统的各种细胞(例如，效应细胞)和补体系统的组分 C1q 的结合。V_H 和 V_L 区还可被细分为具有高变性的区域(称为互补决定区(CDR))，其间散布有较保守的称为骨架区（FR）的区域。各 V_H 和 V_L 由按下列顺序：FR1、CDR1、FR2、CDR2、FR3、CDR3、FR4 从氨基末端至羧基末端排列的 3 个 CDR 和 4 个 FR 组成。各重链/轻链对的可变区(V_H 和 V_L)分别形成抗体结合部位。氨基酸至各区域或结构域的分配遵循 Kabat Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and 1991))，或 Chothia & Lesk (1987) J. Mol. Biol. 196:901-917; Chothia 等人 (1989) Nature 342:878-883 的定义。术语“抗体”不受任何特定的产生抗体的方法限制。例如，其包括，特别地，重组抗体、单克隆抗体和多克隆抗体。抗体可以是不同类型的抗体，例如，IgG (例如，IgG1，IgG2，IgG3 或 IgG4 亚型)，IgA1，IgA2，IgD，IgE 或 IgM 抗体。

在本发明中，所述 IgG 重链恒定区包括 IgG1、 IgG2、IgG3 或 IgG4。在本发明的实施方案中，所述 IgG 重链恒定区为 IgG1 型。

在本发明中，所述 κ 轻链恒定区包括各种同种异型，如 Km1、 Km1,2 或 Km3。

关于抗体的氨基酸序列

在本发明的实施方案中，抗表皮生长因子受体抗体的重链可变区氨基酸序列是 SEQ ID NO:1。在本发明的实施方案中，抗表皮生长因子受体抗体的轻链可变区氨基酸序列是 SEQ ID NO:2。

另一方面，本发明所述的抗体的重链可变区的氨基酸序列与 SEQ ID NO:1 的序列相似性至少达 70%，优选是至少 75%，优选是至少 80%，优选是 85%，再优选是至少 90%，最好的是至少 95%。

另一方面，本发明所述的抗体的轻链可变区的氨基酸序列与 SEQ ID NO:2 的序列相似性至少达 70%，优选是至少 75%，优选是至少 80%，优选是 85%，再优选是至少 90%，最好的是至少 95%。

在本发明的实施方案中，抗表皮生长因子受体抗体的重链和轻链可变区的 CDR 的氨基酸序列确定如下：

重链的 CDR1、CDR2 和 CDR3 的氨基酸序列分别为 SEQ ID NO: 5-7; 轻链的 CDR1、CDR2 和 CDR3 的氨基酸序列分别 SEQ ID NO: 12-14。

另一方面，抗表皮生长因子受体抗体的重链的 CDR 含有的氨基酸序列可能是在 SEQ ID NO: 5-7 上出现一个或多个氨基酸的突变或增添或缺失。

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优选的是，突变、添加或缺失的氨基酸不超过 3 个氨基酸。更优选的是，突变、添加或缺失的氨基酸不超过 2 个氨基酸。最优选的是，突变、添加或缺失的氨基酸不超过 1 个氨基酸。

另一方面，抗表皮生长因子受体抗体的轻链的 CDR 含有的氨基酸序列可能是在 SEQ ID NO: 12-14 上出现一个或多个氨基酸的突变、增添或缺失。优选的是，突变、添加或缺失的氨基酸不超过 3 个氨基酸。更优选的是，突变、添加或缺失的氨基酸不超过 2 个氨基酸。最优选的是，突变、添加或缺失的氨基酸不超过 1 个氨基酸。

在本发明的实施方案中，抗表皮生长因子受体抗体的重链和轻链可变区的 FR 的氨基酸序列确定如下：

重链可变区 FR1、FR2、FR3、FR4 的序列分别为 SEQ ID NO: 8-11。轻链可变区 FR1、FR2、FR3、FR4 的序列分别为 SEQ ID NO: 15-18。

另一方面，抗表皮生长因子受体抗体的重链或轻链可变区 FR 的氨基酸序列可能是在 SEQ ID NO: 8-11、SEQ ID NO: 15-18 上出现一个或多个氨基酸的突变、增添或缺失。优选的是，突变、添加或缺失的氨基酸不超过 3 个氨基酸。更优选的是，突变、添加或缺失的氨基酸不超过 2 个氨基酸。最优选的是，突变、添加或缺失的氨基酸不超过 1 个氨基酸。

上述抗体或 CDR 区或框架区的氨基酸发生突变、添加或缺失之后的变异体仍然保留特异性结合 EGFR 的能力。

在本发明的实施方案中，抗表皮生长因子受体抗体重链恒定区氨基酸序列是 SEQ ID NO:3。在本发明的实施方案中，抗表皮生长因子受体抗体的轻链恒定区氨基酸序列是 SEQ ID NO:4。

另一方面，本发明所述的抗体的重链恒定区的氨基酸序列与 SEQ ID NO:3 的序列相似性至少达 70%，优选是至少 75%，优选是至少 80%，优选是 85%，再优选是至少 90%，最好的是至少 95%，如 96%、97%、98%、99%。

另一方面，本发明所述的抗体的轻链恒定区的氨基酸序列与 SEQ ID NO:4 的序列相似性至少达 70%，优选是至少 75%，优选是至少 80%，优选是 85%，再优选是至少 90%，最好的是至少 95%，如 96%、97%、98%、99%。

本发明所述的单抗变异体可以通过传统的基因工程方法获得。本领域的技术人员完全知晓利用核酸突变改造 DNA 分子的方法。另外，编码重链和轻链变异体的核酸分子也可以通过化学合成获得。

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在本发明中，用于确定序列同一性（同源性）和序列相似性百分数的算法是例如 BLAST 和 BLAST 2.0 算法，它们分别描述在 Altschul 等（1977）Nucl. Acid. Res. 25: 3389-3402 和 Altschul 等（1990）J. Mol. Biol. 215: 403-410。采用例如文献中所述或者默认参数，BLAST 和 BLAST 2.0 可以用于确定本发明的氨基酸序列同一性百分数。执行 BLAST 分析的软件可以通过国立生物技术信息中心为公众所获得。

在本发明中，所述与氨基酸序列具有至少 70％的序列同一性的氨基酸序列包括与所述氨基酸序列基本同一的多肽序列，例如当采用本文所述方法（例如采用标准参数的 BLAST 分析）时，与本发明多肽序列相比含有至少 70％序列同一性、优选至少 75％、80％、85％、86％、87％、88％、89％、90％、91%、92%、93%、94%、95%、96%、97%、98%、99％或更高的序列同一性的那些序列。

在本发明中，用于抗体药物偶联物的毒素包括白喉毒素 A 链、白喉毒素的非结合活性片段、外毒素 A 链（来自铜绿假单胞菌(Pseudomonas aeruginosa)）、蓖麻毒蛋白(ricin)A 链、相思豆毒蛋白(abrin)A 链、蒴莲根毒蛋白(modeccin)A 链、α-帚曲霉素(sarcin)、油桐(Aleutites fordii)毒蛋白、香石竹(dianthin)毒蛋白、美洲商陆(Phytolacaamericana)毒蛋白(PAPI、PAPII 和 PAP-S)、苦瓜(Momordica charantia)抑制物、麻疯树毒蛋白(curcin)、巴豆毒蛋白(crotin)、肥皂草(sapaonaria officinalis)抑制物、白树毒蛋白(gelonin)、丝林霉素(mitogellin)、局限曲菌素(restrictocin)、酚霉素(phenomycin)、依诺霉素(enomycin)、和单端孢菌素(trichothecenes)。参见例如 1993 年 10 月 28 日公布的 WO 93/21232。

多种放射性核素可用于生成抗体药物偶联物。例子包括 ^{212}Bi、^{131}I、^{131}In、^{90}Y、和 ^{186}Re。

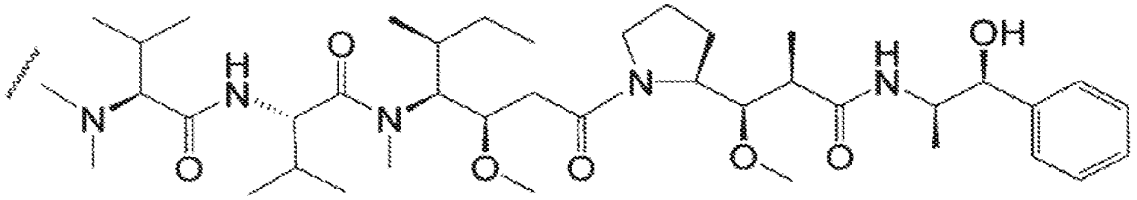
抗体和细胞毒剂的偶联物可使用多种双功能蛋白质偶联剂来制备，诸如 N-琥珀酰亚氨基 3-(2-吡啶基二硫代)丙酸酯(SPDP)，亚氨基硫烷(IT)，亚氨酸酯(诸如盐酸己二酰亚氨酸二甲酯)、活性酯类(诸如辛二酸二琥珀酰亚氨基酯)、醛类(诸如戊二醛)、双叠氮化合物(诸如双(对-叠氮苯甲酰基)己二胺)、双重氮衍生物(诸如双(对-重氮苯甲酰基)-乙二胺)、二异硫氰酸酯(诸如甲苯 2，6-二异氰酸酯)、和双活性氟化合物(诸如 1，5-二氟-2，4-二硝基苯)的双功能衍生物。例如，可如 Vitetta 等在(1987)Science，238: 1098 中所述制备的含蓖麻毒的免疫毒素。碳-14 标记的 1-异硫氰酸苄基-3-甲基二亚乙基三胺五乙

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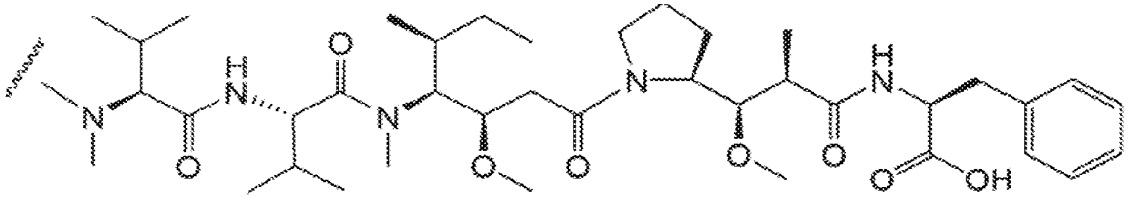
酸(MX-DTPA)是用于将放射性核苷酸与抗体偶联的例示性螯合剂(WO94/11026)。 本发明还涵盖抗体和一种或多种小分子毒素(诸如加利车霉素(calicheamicin)、美登素生物碱(maytansinoid)、多拉司他汀(dolastatin)、 auristatin、单端孢霉素(trichothecene)、和 CC 1065 及这些毒素的具有毒素活性的衍生物的偶联物。

在有些实施方案中，抗体药物偶联物包含与多拉司他汀(dolastatin)或多拉司他汀肽类似物和衍生物 auristatin(美国专利 No.5,635,483; 5,780,588)偶联的抗表皮生长因子受体抗体。多拉司他汀和 auristatin 已经显示出干扰微管动力学、GTP 水解、及核和细胞分裂等活性(Woyke 等(2001)Antimicrob.Agents and Chemother.45(12): 3580-3584)且具有抗癌(US 5,663,149) 和抗真菌活性(Pettit 等(1998) Antimicrob.Agents Chemother.42: 2961-2965)。多拉司他汀或 auristatin 药物模块可经由肽药物模块的 N(氨基)末端或 C(羧基)末端附着于抗体(WO 02/088172)。

在本发明中，其中 MMAE 的结构为:



在本发明中，其中 MMAF 的结构为:



在本发明中，作为细胞毒剂的酶可以为具有核酸降解活性的化合物(例如核糖核酸酶或 DNA 内切核酸酶，诸如脱氧核糖核酸酶; DNA 酶)。

在本发明中，药物载荷(loading)由 p 表示，即式 I: Ab-(L-D)$_p$ 的分子中每个抗体的平均药物模块（即细胞毒剂）数。药物载荷的范围可以为每个抗体 1-20 个药物模块(D)。通式 I 的 ADC 包括偶联有一定范围(1-20 个)药物模块的抗体的集合。来自偶联反应的 ADC 制备物中每个抗体的平均药物模块数可以通过常规手段来验证，诸如质谱、ELISA 测定法、和 HPLC。还可以测定 ADC 在 p 方面的定量分布。在有些情况中，将 p 为某数值的同质 ADC

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从具有其它药物载荷的 ADC 中分离、纯化和验证可以通过诸如反相 HPLC 或电泳的手段来实现。

对于有些抗体-药物偶联物，p 可能受到抗体上附着位点数目的限制。例如，若附着位点是半胱氨酸硫醇，抗体可能只有一个或数个半胱氨酸硫醇基，或者可能只有一个或数个有足够反应性的硫醇基，可附着接头。在某些实施方案中，较高的药物载荷，例如 $p > 5$，可引起某些抗体-药物偶联物的聚集、不溶性、毒性、或丧失细胞通透性。

在某些实施方案中，本发明 ADC 的药物载荷范围为 1 到约 8；约 2 到约 6；约 3 到约 5；约 4 到约 5；约 3.5 到 4.5；约 4。事实上，对于某些 ADC 已经显示了每个抗体的药物模块的最佳比率可以为小于 8，可以为约 2 到约 5。参见 US2005-0238649A1(完整收入本文作为参考)。

在某些实施方案中，在偶联反应中少于理论最大值的药物模块偶联至抗体。抗体可包含例如赖氨酸残基，其不与药物-接头中间物或接头试剂起反应。一般而言，抗体不包含许多游离的和反应性的半胱氨酸硫醇基，其可连接药物模块；事实上，抗体中的大多数半胱氨酸硫醇基以二硫桥形式存在。在某些实施方案中，可以在部分或完全还原性条件下用还原剂诸如二硫苏糖醇(DTT)或三羰基乙基膦(TCEP)还原抗体以产生反应性半胱氨酸硫醇基。在某些实施方案中，将抗体置于变性条件以暴露反应性亲核基团，诸如赖氨酸或半胱氨酸。

ADC 的载荷(药物/抗体比率)可以以不同方式来控制，例如通过：(i)限制药物-接头中间物或接头试剂相对于抗体的摩尔数，(ii)限制偶联反应的时间或温度，(iii)半胱氨酸硫醇修饰的部分或限制还原性条件，(iv)通过重组技术对抗体的氨基酸序列进行工程改造，使得半胱氨酸残基的数目和位置为了控制接头-药物附着的数目和/或位置而进行改变。应当理解，若超过一个亲核基团与药物-接头中间物或者与接头试剂和接下来的药物模块试剂起反应，则所得产物是具有一个或多个药物模块附着于抗体的 ADC 化合物混合物。可以通过对抗体特异性和对药物特异性的双重 ELISA 抗体测定法自混合物计算每个抗体的平均药物数。混合物中的各种 ADC 分子可以通过质谱来鉴定，并通过 HPLC 来分离，例如疏水相互作用层析。在某些实施方案中，可以通过电泳或层析从偶联混合物中分离具有单一载荷值的同质 ADC。

在本发明中，抗体药物偶联物的药学上可接受的盐包括无机酸、羧酸和磺酸的酸加成盐，例如以下酸的盐：盐酸、氢溴酸、硫酸、磷酸、甲烷磺酸、乙烷磺酸、苯磺酸、甲苯磺酸、萘二磺酸、乙酸、三氟乙酸、丙酸、乳酸、

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酒石酸、苹果酸柠檬酸、富马酸、马来酸和苯甲酸。

本发明的抗体药物偶联物的药学上可接受的盐还包括常规碱的盐，例如（仅为示例性并优选）碱金属盐（例如钠盐和钾盐）、碱土金属盐（例如钙盐和镁盐）和衍生自氨或含有1-16个碳原子的有机胺的铵盐，所述有机胺例如（仅为示例性并优选）乙胺、二乙胺、三乙胺、乙基二异丙胺、单乙醇胺、二乙醇胺、三乙醇胺、二环己胺、二甲基氨基乙醇、普鲁卡因、二苯甲酰胺、N- 甲基哌啶、N- 甲基吗啉、精氨酸、赖氨酸和1，2- 乙二胺。

在本发明中，溶剂合物表示这些形式的本发明的抗体药物偶联物：所述抗体药物偶联物通过与溶剂分子配位而形成的固态或液态形式的复合物。水合物是溶剂合物的一种具体形式，其具有配位的水分子。在本发明中，水合物是优选的溶剂合物。

可以优选地使用本发明的抗体药物偶联物进行治疗的与EGFR相关的肿瘤包括过表达EGFR 的肿瘤、呼吸道肿瘤（例如小细胞癌和非小细胞癌、支气管癌），其中特别优选非小细胞肺癌；消化道（例如食道、胃、胆囊、小肠、大肠、直肠）的肿瘤，其中特别优选肠肿瘤；内分泌腺和外分泌腺（例如甲状腺和甲状旁腺、胰腺和唾液腺）的肿瘤，其中特别优选胰腺肿瘤；头颈区域（例如喉、下咽、鼻咽、口咽、唇、口腔、舌和食道）的肿瘤；和/ 或神经胶质瘤。

在本发明中，EGFR过表达是指与正常上皮细胞表面的EGFR表达水平相比，EGFR的表达水平提高；其具体又可以分为高表达、中度表达和低表达，例如DiFi细胞是EGFR高表达细胞株，LoVo细胞是EGFR中度表达的细胞株，而HT-29细胞是EGFR低表达细胞株 （Wild, R., et al., Mol. Cancer Rher 2006:5(1), p104-113, Cetuximab preclinical antitumor activity (monotherapy and combination based) is not predicted by relative total or activated epidermal growth factor receptor tumor expression levels）。

本发明的抗体药物偶联物可以与已知的用于治疗肿瘤的化疗药物联用，所述化疗药物例如为阿霉素(Adriamycin)、环磷酰胺和紫杉烷类[紫杉醇(Taxol)和多西他赛(Taxotere)]、卡培他滨(Xeloda)、吉西他滨(Gemzar)、长春瑞滨(Navelbine)、他莫昔芬、芳香酶抑制剂（瑞宁得、弗隆、阿诺新）、5-FU 加亚叶酸、伊立替康(camptosar)、奥沙利铂、顺铂、卡铂、雌莫司汀、米托蒽醌(Novantrone)、泼尼松、长春新碱(Oncovin)等，或它们的组合。

在本发明中，“治疗”指试图改变所治疗个体或细胞的自然进程的临床干预，可以是为了预防或在临床病理学的进程中进行。治疗的期望效果包括预防疾病的发生或复发、缓解症状、削弱疾病的任何直接或间接病理学后果、

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预防转移、减缓疾病进展的速率、改善或减轻疾病状态、及免除或改善预后。在有些实施方案中，本发明的抗体或抗体药物偶联物用于延迟疾病或病症的发生，或用于减缓疾病或病症的进展。用于评估疾病的成功治疗和改善的上述参数可以容易的通过内科医师所熟悉的常规规程来测量。对于癌症治疗，可通过例如评估疾病进展前时间 (TTP)和/或测定反应率(RR)来测量功效。

在本发明中，“受试者”指脊椎动物。在某些实施方案中，脊椎动物指哺乳动物。哺乳动物包括，但不限于，牲畜(诸如牛)、宠物(诸如猫、犬、和马)、灵长类动物、小鼠和大鼠。在某些实施方案中，哺乳动物指人。

在本发明中，“有效量”指在必需的剂量和时间上有效实现期望的治疗或预防效果的量。本发明的物质/分子的“治疗有效量”可根据诸如个体的疾病状态、年龄、性别和体重及该物质/分子在个体中引发期望应答的能力等因素而变化。治疗有效量还涵盖该物质/分子的治疗有益效果胜过任何有毒或有害后果的量。“预防有效量”指在必需的剂量和时间上有效实现期望的预防效果的量。通常而非必然，由于预防剂量是在疾病发作之前或在疾病的早期用于受试者的，因此预防有效量会低于治疗有效量。在癌症的情况中，药物的治疗有效量可减少癌细胞数；缩小肿瘤体积；抑制(即一定程度的减缓，优选停止)癌细胞浸润到周围器官中；抑制(即一定程度的减缓，优选停止)肿瘤转移；一定程度的抑制肿瘤生长；和/或一定程度的减轻与癌症有关的一种或多种症状。

对于疾病的预防或治疗，本发明的抗体药物偶联物的适宜剂量(在单独地或联合一种或多种其它别的治疗剂诸如化疗剂地使用时)会取决于待治疗疾病的类型、抗体药物偶联物的类型、疾病的严重程度和进程、施用抗体药物偶联物是出于预防还是治疗目的、先前的疗法、患者的临床病史和对抗体药物偶联物的反应性、及主治医师的判断。合适的是，一次性或通过一系列治疗将抗体药物偶联物施用于患者。根据疾病的类型和严重程度，施用于患者的初始候选剂量可以是约 1μg/kg 至 100mg/kg(例如 0.1mg/kg-20mg/kg)抗体药物偶联物，例如或是通过一次或多次分开施药或是通过连续输注。根据上文所述因素，典型日剂量的范围可以是约 1μg/kg 至 100mg/kg 或更多。对于持续数天或更长时间的重复施药，根据状况，通常持续治疗直至疾病症状发生期望的抑制。抗体药物偶联物的例示剂量的范围可以是约 0.05mg/kg 至约 10mg/kg。如此，可以对患者施用一剂或多剂约 0.5mg/kg、2.0mg/kg、4.0mg/kg 或 10mg/kg(或其任意组合)的抗体药物偶联物。此类剂量可间歇施用，例如每周或每三周(例如使得患者接受约 2 剂至约 20 剂，或例如约 6 剂抗体药物

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偶联物)。可施用一剂较高的初始加载剂量，后续一剂或多剂较低剂量。这种疗法的进程易于通过常规技术和测定法来监测。“长期”施用指与短期模式相反，以连续模式施用药剂，从而将初始治疗效果(活性)维持较长一段时间。“间歇”施用指不是无间断连续进行的治疗，而是本质上周期性的。“联合”一种或多种其它治疗剂的施用包括同时(共同)施用和任意次序的序贯施用。

“药学上可接受的载体”在用于本发明时包括药学可接受的载体、赋形剂或稳定剂，它们在所采用的剂量和浓度对暴露于其的细胞或哺乳动物是无毒的。通常，生理学可接受的载体是 pH 缓冲水溶液。生理学可接受载体的例子包括缓冲剂，诸如磷酸盐、柠檬酸盐和其它有机酸；抗氧化剂，包括抗坏血酸；低分子量(少于约 10 个残基)多肽；蛋白质，诸如血清清蛋白、明胶或免疫球蛋白；亲水性聚合物，诸如聚乙烯吡咯烷酮；氨基酸，诸如甘氨酸、谷氨酰胺、天冬酰胺、精氨酸或赖氨酸；单糖、二糖和其它碳水化合物，包括葡萄糖、甘露糖，蔗糖，海藻糖或糊精；螯合剂，诸如 EDTA；糖醇，诸如甘露醇或山梨醇；成盐反荷离子，诸如钠；和/或非离子表面活性剂，诸如 TWEEN™、聚乙二醇(PEG)和 PLURONICS™。

在本发明中，KRAS 基因与 K-RAS 基因具有相同的含义，其为 RAS 基因家族成员之一，编码 K-ras 蛋白，与多种肿瘤的生成、增殖、迁移、扩散以及血管生成有关。其常见的突变位点为 K-RAS 基因 2 号外显子的 12 号密码子和 13 号密码子，3 号外显子的 61 号密码子，其中有 7 个突变热点：G12C、G12R、G12S、G12V、G12D、G12A、G13V/D.这 7 种突变占到了 90%以上。在本发明的一个实施方案中，所述肿瘤为与 EGFR 过表达相关的 KRAS 基因突变的肿瘤。

在本发明中，BRAF（v-raf murine sarcoma viral oncogene homologB1）基因是一种原癌基因，是 RAF 家族成员之一。大约 8％的人类肿瘤发生 BRAF 突变，BRAF 基因突变的绝大部分突变形式为 BRAFV600E 突变，该突变导致下游 MEK／ERK 信号通路持续激活，对肿瘤的生长增殖和侵袭转移至关重要。在本发明的一个实施方案中，所述肿瘤为与 EGFR 过表达相关的 BRAF 基因突变的肿瘤。

在本发明中，20 种常规氨基酸和其缩写遵从常规用法。参见 Immunology-A Synthesis (第 2 版, E.S. Golub 和 D.R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991))，其通过引用合并入本文。

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附图说明

图 1 测定抗体药物偶联物的药物/抗体比例的 HIC-HPLC 图。

图 2 单抗及抗体药物偶联物的体外细胞活性测定，其中○代表 BA03 单克隆抗体，▲代表 MYK-3 抗体药物偶联物。

图 3 MYK-3 对结肠癌细胞 HT-29 的生长抑制活性，其中○代表 BA03 单克隆抗体，▲代表 MYK-3 抗体药物偶联物。

图 4 MYK-3 对脑胶质瘤癌细胞 U87-MG 的生长抑制活性，其中○代表 BA03 单克隆抗体，▲代表 MYK-3 抗体药物偶联物。

图 5 MYK-3 对肺癌细胞 A549 的生长抑制活性，其中○代表 BA03 单克隆抗体，▲代表 MYK-3 抗体药物偶联物。

图 6 MYK-3 对 KRAS 突变体结肠癌细胞 LoVo 的生长抑制活性，其中◇代表爱必妥单克隆抗体，▲代表 MYK-3 抗体药物偶联物。

图 7 单抗及抗体药物偶联物对小鼠 HT-29 结肠癌移植瘤体积的影响。图中数据用平均值±标准差表示；与缓冲液对照组相比，*表示 $P<0.05$，**表示 $P<0.01$，***表示 $P<0.001$。

图 8 单抗及抗体药物偶联物对小鼠 HT-29 结肠癌移植瘤模型的体重的影响。

图 9 MYK-3、单抗 BA03 和 BA03+等摩尔数 vcMMAE 对结直肠癌细胞 DiFi 的生长抑制活性，其中，□代表 MYK-3 的单抗组分 BA03，Δ代表单抗组分 BA03 加上同等于 MYK-3 载药摩尔数的 vcMMAE，◆ 代表 MYK-3。

图 10 MYK-3 对裸鼠中 KRAS 突变体结肠癌细胞 LoVo 的移植瘤生长抑制活性。

图 11 BA03-MCC-MMAE、BA03-MC-MMAE 和 MYK-3 的 HIC-HPLC 分析图谱。

图 12 MYK-3 与 BA03-MC-MMAE、BA03-MCC-MMAE 的体外细胞活性比较，其中，□代表 BA03-MC-MMAE，Δ代表 BA03-MCC-MMAE，◆代表 MYK-3。

具体实施方式

下面将结合实施例对本发明的实施方案进行详细描述，但是本领域技术人员将会理解，下列实施例仅用于说明本发明，而不应视为限定本发明的范围。实施例中未注明具体条件者，按照常规条件或制造商建议的条件进行。所用试剂或仪器未注明生产厂商者，均为可以通过市购获得的常规

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产品。

本发明中的抗体 BA03 即中国发明专利申请 CN 103772504A 中的 BA03，其制备方法参考该专利申请中的实施例 3，该抗体各部分的序列如下：

重链可变区序列为：

QVQLQESGPGLVKPSETLSLTCTVSGFSLSNYDVHWVRQAPGKGLEWLGVIWSGGNTDYNTPFTSRLTISVDTSKNQFSLKLSSVTAADTAVYYCARALDYYDYEFAYWGQGTLVTVSS（SEQ ID NO: 1）。

其中下划线部分分别为 CDR1（SEQ ID NO: 5）、CDR2（SEQ ID NO: 6）、CDR3（SEQ ID NO: 7）；

未下划线部分分别为 FR1（SEQ ID NO: 8）、FR2（SEQ ID NO: 9）、FR3（SEQ ID NO: 10）、FR4（SEQ ID NO: 11）。

轻链可变区序列为：

EIVLTQSPDFQSVTPKEKVTITCRASQSIGTNIHWYQQKPDQSPKLLIKYASESISGIPSRFSGSGSGTDFTLTINSLEAEDAATYYCQQNNEWPTSFGQGTKLEIK（SEQ ID NO: 2）。

其中下划线部分分别为 CDR1（SEQ ID NO: 12）、CDR2（SEQ ID NO: 13）、CDR3（SEQ ID NO: 14）；

未下划线部分分别为 FR1（SEQ ID NO: 15）、FR2（SEQ ID NO: 16）、FR3（SEQ ID NO: 17）、FR4（SEQ ID NO: 18）。。

重链恒定区序列为：

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK（SEQ ID NO: 3）。

轻链恒定区序列为：

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC（SEQ ID NO: 4）。

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实施例 1 抗体药物偶联物的制备方法

取 10 毫克的 BA03 抗体，使用 15mL 的 30KD 超滤装置置换到还原缓冲液 (25mM 硼酸钠, pH8.0, 25mM NaCl, 5 mM EDTA)中，共置换三次；终体积约为 1mL,转移至新的 Eppendorf 离心管（称重）中，并称重;检测蛋白浓度，计算蛋白总量。向抗体中加入 2.5 倍摩尔数的 DTT，室温保温 2 小时，连续混匀；使用 15ml 的 30KD 超滤装置置换到偶联缓冲液 （50mM Tris, pH7.2, 150mM NaCl, 5 mM EDTA）中，共置换三次。取浓缩液，以 A280 测定蛋白浓度，并称重，计算蛋白总量；取 10μl 样品，以 Ellman's 方法测定自由巯基数;

并以下列公式计算其自由巯基的摩尔浓度:

$$C_{thiol}=\frac{A412\times 11.2}{b\times 14150}\ (M)$$

b: 比色皿光路长度（通常 1cm）。

根据自由巯基的摩尔浓度和总蛋白溶液体积计算自由巯基摩尔数。

向还原后的抗体中加入 1.1 倍于自由巯基摩尔数的 vc-MMAE （购上海皓元化学科技有限公司，货号 HY-15575）（溶于 DMSO），混匀后室温反应 2 小时，间断混匀。向反应体系中加入 20 倍于所投入之 vc-MMAE 摩尔数的 N-乙酰半胱氨酸于反应液中，混匀，静置 5 分钟。使用 15ml 的 30KD 超滤装置置换到偶联物储存液（20mM 柠檬酸钠(Na-citrate), 0.3% NaCl, 5% 海藻糖（ Trehalose ）, 0.05% TWeen-80, pH6.0 ）中，共置换三次，即得到抗体药物偶联物 MYK-3; 样品于 4℃保存。

药物/抗体比例的测定:

将制备好的抗体药物偶联物 MYK-3 经过 HIC-HPLC 分析（Jun Ouyang, Drug-To-Antibody(DAR) Ratio and Drug Distributionby Hydrophobic Interaction Chromatography and Reverse Phase High Performance Chromatography, Laurent Ducry(ed.), Antibody Drug Conjugates, Chapter 17, Methods in Molecular Biology, Vol 1045, p275-283 ）以测定药物/抗体比例（drug antibody ratio， DAR），见图 1，根据图谱峰面积计算得到平均载药数 DAR 为 4.1。

实施例 2 抗体药物偶联物 MYK-3 体外细胞活性的测定

细胞活性检测方法:

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1.1 复苏后的细胞株经过 3-4 代细胞传代之后，先倒去培养基，用 5mL DPBS 润洗一遍，再用 3mL 胰蛋白酶消化细胞，分别用培养基重悬，离心机离心，弃上清，再用培养基再次重悬，取出 0.5mL 用细胞计数仪计数。在 96 孔细胞培养板上进行铺板（DiFi 细胞以 10000 个细胞/孔，HT-29 细胞以 5000 个细胞/孔, A549 细胞以 2000 个细胞/孔，U87-MG 细胞以 3000 个细胞/孔， LoVo 细胞以 4000 个细胞/孔）， 培养 24 小时后，加入系列稀释浓度的单克隆抗体 BA03 以及抗体药物偶联物 MYK-3，保温 72 小时，之后每孔加入 20μl CCK8 显色试剂，在波长 450-650nm 用酶标仪检测 OD450-650，并进行四参数拟合。

体外细胞活性实验结果:

以下各细胞株购自中国科学院上海细胞生物学研究所。

在 EGFR 高表达的 DiFi 细胞（人结直肠癌细胞）中的活性： MYK-3 比单抗 BA03 有显著增高的细胞生长抑制活性， EC50 降低了大约 10 倍（BA03 的 EC50 是 51.9 ng/ml， MYK-3 的 EC50 是 5.1 ng/ml）， 如图 2 所示。

在 EGFR 中度表达和低度表达的其它肿瘤细胞中的活性： MYK-3 相对于单抗本身对 EGFR 中度表达和低度表达的癌细胞（人结肠癌细胞 HT29、人肺癌细胞 A549、人脑星形胶质母细胞瘤细胞 U87-MG）也显示了明显的细胞生长抑制活性（如图 3、图 4、图 5 所示), 其中 HT-29 的 EC50 是 611 ng/ml, A549 的 EC50 是 28.3 μg/ml，U87-MG 的 EC50 是 5.3 μg/ml。

另外，我们还测试了在 EGFR 中度表达的 KRAS 突变体结肠癌细胞 LoVo (Dunn EF, Ilda M, Myers RA, Hintz KA, Campbell DA, Armstrong EA, Li C and Wheeler DL. Dasatinib sensitizes KRAS mutant colorectal tumors to cetruximab. Oncogene 2011; 30:561-574)中的活性，发现 MYK-3 对 KRAS 突变体结肠癌细胞 LoVo 显示了明显的肿瘤生长抑制活性(如图 6 所示，EC50 是 3.2μg/ml)，而 BA03 单独使用时对该细胞株几乎没有抑制活性。

实施例 3 小鼠体内移植瘤实验

小鼠体内移植瘤实验方法:

HT-29 结肠癌细胞是 EGFR 低表达并带有 BRAF 突变的细胞株，目前已在市场上销售的治疗结直肠癌的 EGFR 靶向单抗爱必妥(Erbitux) 对 HT-29 细胞株没有生长抑制活性。

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HT-29 细胞移植模型：收集对数生长期的肿瘤细胞，计数后重悬于1×PBS，调整细胞悬液浓度至 3×10^7/ml。用 1ml 注射器（4 号针头）在裸鼠右侧背部皮下接种肿瘤细胞，3×10^6/0.1ml/鼠，当肿瘤体积达到 150-200mm^3 后用随机区组法分组，每组 8 只小鼠，保证各组间肿瘤体积和小鼠体重均一。各组肿瘤体积的均值与所有实验动物肿瘤体积的均值差异不超过±10％。 尾静脉给药，每四天给一次药（第 1、5、9、13 天），一共给药 4 次，定期测量肿瘤体积和小鼠体重。每一个给药组分别有 8 只小鼠。

小鼠移植瘤实验结果

小鼠 HT-29 结肠癌移植瘤实验：实验共分五组， 包括缓冲液组（20 mM 柠檬酸钠，0.3%氯化钠，5%海藻糖，0.05%吐温 80， pH6）， BA03 单抗组（5mg/kg）、MYK-3 组（1mg/kg）、MYK-3 组（5mg/kg）和非结合 ADC 组（5mg/kg）（人源 IgG-vcMMAE 偶联物，其中 IgG 是从健康人血清纯化的 IgG，该偶联物制备方法与 MYK-3 相同）。MYK-3 给药的小鼠移植肿瘤体积与对照组比较显著降低，显示了明显的抗肿瘤生长效应（图 7）。在第 18 天，MYK-3 在 5mg/Kg 的给药剂量组与缓冲液组相比肿瘤生长抑制率达到 54%，与单抗 BA03 的同等剂量组相比肿瘤生长抑制率达到 46%， 与非结合 ADC 相比肿瘤生长抑制率达到 42%。

小鼠体重：MYK-3 给药的小鼠体重与对照组比较没有变化（见图 8），说明 MYK-3 没有使小鼠体重降低的毒性作用。

实施例 4 MYK-3、单抗 BA03 和 BA03+等摩尔数 vcMMAE 对结直肠癌细胞 DiFi 的生长抑制活性

按照实施例 2 方法检测了 MYK-3、单抗 BA03 和 BA03+等摩尔数 vcMMAE 对结直肠癌细胞 DiFi 的生长抑制活性，实验结果见图 9，其中的 EC50 值分别为 8.4 ng/mL, 65.8 ng/mL 和 68.2 ng/mL。

从图中可以看出，单抗 BA03 在 EGFR 高表达的结直肠癌细胞 DiFi 中显示了一定的生长抑制活性；并且 BA03 加上同等于 MYK-3 载药摩尔数的游离 vcMMAE 与单独的 BA03 活性比较没有明显区别；然而，BA03 与 vcMMAE 偶联形成的 MYK-3 作为 ADC 分子对 DiFi 结直肠癌细胞的生长抑制活性大大高于单抗 BA03 本身以及 BA03 加上同等于 MYK-3 载药摩尔数的游离 vcMMAE 的活性，EC50 提高了大约 8 倍。

实施例 5 MYK-3 对裸鼠中 KRAS 突变体结肠癌细胞 LoVo 的移植瘤生

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长抑制活性

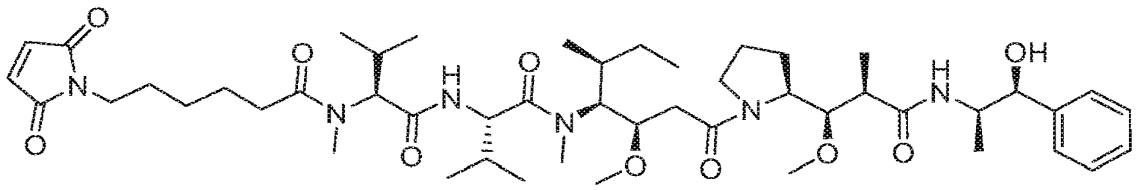
按照实施例 3 的模型构建（以 2×10^6/0.1ml/鼠接种肿瘤细胞）和给药方法检测 MYK-3 对裸鼠中 KRAS 突变体结肠癌细胞 LoVo 的移植瘤生长抑制活性，实验共分六组，包括稀释缓冲液组（20 mM 柠檬酸钠，0.3%氯化钠，5%海藻糖，0.05%吐温 80， pH6），爱必妥单抗组（3mg/kg）、MYK-3 组（0.3mg/kg、1mg/kg、3mg/kg 三个剂量）和非结合对照 ADC 组（3mg/kg），其中非结合对照 ADC 代表非结合的同等载药量的 ADC 对照（non-binding ADC control,为 anti-CD20 mAb-vcMMAE），该偶联物制备方法与 MYK-3 相同。实验结果见图 10。

从图中可以看出，MYK-3 在 3 mg/Kg 剂量下表现出对 LoVo 细胞肿瘤生长的完全抑制，并且 MYK-3 在 1 mg/Kg 已显示出比上市药品 Erbitux 在 3 mg/Kg 剂量下还更强的活性。

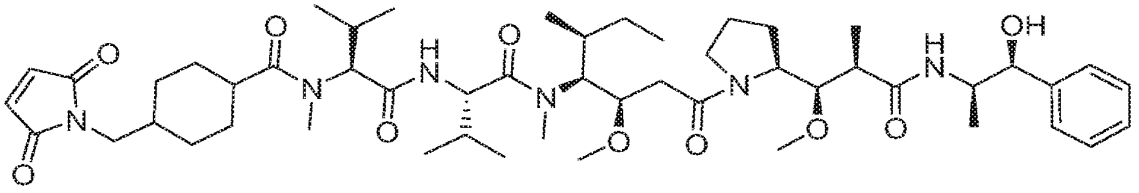
实施例 6 MYK-3 与 BA03-MC-MMAE、 BA03-MCC-MMAE 的体外细胞活性比较

MC-MMAE 和 MCC-MMAE 的结构如下所示:

MC-MMAE:



MCC-MMAE:



MC-MMAE 和 MCC-MMAE 的制备方法参见实施例 1。

药物/抗体比例的测定： 使用 HIC-HPLC 分析方法测定药物/抗体比例，具体参见实施例 1，测得的 MYK-3（即为 BA03-vcMMAE），BA03-MC-MMAE

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和 BA03-MCC-MMAE 的药物/抗体比例均为 3.9，参见图 11。

体外细胞活性实验方法参见实施例 2；简而言之，取一定数量的 DiFi 细胞株，接种于 96 孔板中，培养 24 小时后加入不同浓度梯度稀释的 BA03-MC-MMAE，BA03-MCC-MMAE，MYK-3 三个样品。再培养 96 小时，加入 CCK-8 检测试剂显色，在酶标仪上读取 96 孔板每个孔的 OD 值，检测不同浓度样品的活细胞数量，判断样品对 DiFi 细胞株的增殖抑制效果。实验结果见图 12，其中 BA03-MC-MMAE、BA03-MCC-MMAE 和 MYK-3 的 EC50 值分别为 72.6 ng/mL，71.6 ng/mL 和 9.8 ng/mL。

由图中可见，MYK-3 的细胞增殖抑制活性明显高于 BA03-MC-MMAE 和 BA03-MCC-MMAE 的活性；事实上，BA03-MC-MMAE 和 BA03-MCC-MMAE 的细胞增殖抑制活性与单抗 BA03 本身的活性相似。这说明使用同样的单抗和细胞毒小分子，但使用不同的链接体可以造成 ADC 的活性有显著差异。

尽管本发明的具体实施方式已经得到详细的描述，本领域技术人员将会理解。根据已经公开的所有教导，可以对那些细节进行各种修改和替换，这些改变均在本发明的保护范围之内。本发明的全部范围由所附权利要求及其任何等同物给出。

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权 利 要 求 书

1. 抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其包含与细胞毒剂共价连接的抗表皮生长因子受体抗体。

2. 权利要求 1 的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的抗表皮生长因子受体抗体包括重链和轻链，其中重链可变区 CDR1、CDR2、CDR3 分别包含如 SEQ ID NO: 5～7 所示的序列或其突变体，轻链可变区 CDR1、CDR2、CDR3 分别包含如 SEQ ID NO: 12～14 所示的序列或其突变体。

3. 权利要求 2 的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的抗表皮生长因子受体抗体重链可变区 FR1、FR2、FR3、FR4 区分别包含如 SEQ ID NO: 8～11 所示的序列或其突变体。

4. 权利要求 2 的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的抗表皮生长因子受体抗体轻链可变区 FR1、FR2、FR3、FR4 区分别包含如 SEQ ID NO: 15～18 所示的序列或其突变体。

5. 权利要求 1-4 任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的抗表皮生长因子受体抗体的重链恒定区选自人源性 IgG、IgM、IgA、IgD、IgA 恒定区或上述恒定区的突变体。

6. 权利要求 5 的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述 IgG 选自 IgG1、IgG2、IgG3 和 IgG4。

7. 权利要求 5 的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的抗表皮生长因子受体抗体重链恒定区的氨基酸序列包含如 SEQ ID NO: 3 所示的序列，或者包含与 SEQ ID NO: 3 所示序列的同一性大于 70%，例如大于 75%、80%、85%、90%、95%、

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99%的序列。

8. 权利要求 1-7 任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的抗表皮生长因子受体抗体的轻链恒定区为人源性 lambda 恒定区、kappa 恒定区、或上述恒定区的突变体。

9. 权利要求 8 的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的抗表皮生长因子受体抗体轻链恒定区的氨基酸序列包含如 SEQ ID NO: 4 所示的序列，或者包含与 SEQ ID NO: 4 所示序列的同一性大于 70%，例如大于 75%、80%、85%、90%、95%、99%的序列。

10. 权利要求 1-9 任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其具有式 I 所示的结构，

$$Ab-(L-D)_p$$

式 I

其中：

Ab 代表抗表皮生长因子受体抗体；

L 代表接头；

D 代表细胞毒剂；

p 代表 1-9，例如 2-6，例如 3-5。

11. 权利要求1-10任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的细胞毒剂选自化疗药物、毒素、放射性同位素、细胞因子、抗生素、酶、纳米颗粒和生物活性肽。

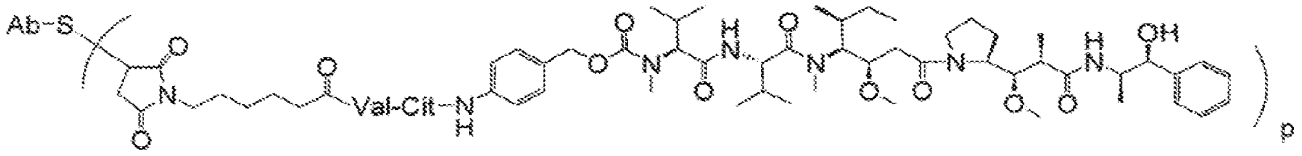
12. 权利要求11的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的细胞毒剂选自Monomethyl auristatin E（MMAE）、 Monomethyl auristatin F（MMAF）、 美登木素生物碱（例如Maytansine DM1、Maytansine DM4）、卡奇霉素(calicheamicin)、duocarmycin MGBA、阿霉素(doxorubicin)、蓖麻毒素、白喉毒素等毒素、I131、白介素类、肿瘤坏死因子、趋化因子和纳米颗粒等，优选为MMAE。

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13. 权利要求 10 的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的接头为可切割的或不可切割的。

14. 权利要求 13 的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的接头选自 6-马来酰亚氨基己酰基(MC)、马来酰亚氨基丙酰基(MP)、缬氨酸-瓜氨酸(val-cit)、丙氨酸-苯丙氨酸(ala-phe)、对氨基苄氧羰基(PAB)、N-琥珀酰亚氨基 4-(2-吡啶基硫代)戊酸酯(SPP)、N-琥珀酰亚氨基 4-(N-马来酰亚氨基甲基)-环己烷-1-羧酸酯(SMCC)、N-琥珀酰亚氨基(4-碘-乙酰基)氨基苯甲酸酯(SIAB)、和 6-马来酰亚氨基己酰基-缬氨酸-瓜氨酸-对氨基苄氧羰基(MC-vc-PAB)，优选为 6-马来酰亚氨基己酰基-缬氨酸-瓜氨酸-对氨基苄氧羰基(MC-vc-PAB)。

15. 权利要求 1-14 任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其为：



其中Ab为抗表皮生长因子受体抗体，p为1-8，例如2-6，例如3-5。

16. 组合物，其含有权利要求1-15任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，任选地，还含有至少一种药学上可接受的载体、稀释剂或赋形剂。

17. 权利要求1-15任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物在制备预防和/或治疗与表皮生长因子受体（EGFR）相关的疾病的药物中的用途。

18. 权利要求16的用途，其中所述的与表皮生长因子受体（EGFR）相关的疾病为与EGFR相关的肿瘤，例如与EGFR过表达相关的肿瘤，例如选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

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19. 权利要求1-15任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物在制备抑制肿瘤血管生成、延缓肿瘤进展、抑制肿瘤生长、抑制肿瘤细胞增殖的药物中的用途。

20. 权利要求19的用途，其中所述的肿瘤选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

21. 权利要求18或20的用途，其中所述的肿瘤为KRAS基因突变的肿瘤，例如为KRAS基因突变的结肠癌、直肠癌、肺癌或胰腺癌。

22. 权利要求18或20的用途，其中所述的肿瘤为BRAF基因突变的肿瘤，例如选自BRAF基因突变的结肠癌、直肠癌和肺癌。

23. 预防和/或治疗与表皮生长因子受体（EGFR）相关的疾病的方法，所述方法包括给予有需要的受试者预防和/或治疗有效量的权利要求1-15任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物。

24. 权利要求23的方法，其中所述的与表皮生长因子受体（EGFR）相关的疾病为与EGFR相关的肿瘤，例如与EGFR过表达相关的肿瘤，例如选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

25. 抑制肿瘤血管生成、延缓肿瘤进展、抑制肿瘤生长、抑制肿瘤细胞增殖的方法，所述方法包括给予有需要的受试者预防和/或治疗有效量的权利要求1-15任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物。

26. 权利要求25的方法，其中所述的肿瘤选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

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27. 权利要求24或26的方法，其中所述的肿瘤为KRAS基因突变的肿瘤，例如为KRAS基因突变的结肠癌、直肠癌、肺癌或胰腺癌。

28. 权利要求24或26的方法，其中所述的肿瘤为BRAF基因突变的肿瘤，例如选自BRAF基因突变的结肠癌、直肠癌和肺癌。

29. 权利要求1-15任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其用于预防和/或治疗与表皮生长因子受体（EGFR）相关的疾病。

30. 权利要求29的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的与表皮生长因子受体（EGFR）相关的疾病为与EGFR相关的肿瘤，例如与EGFR过表达相关的肿瘤，例如选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

31. 权利要求1-15任一项的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其用于抑制肿瘤血管生成、延缓肿瘤进展、抑制肿瘤生长、抑制肿瘤细胞增殖。

32. 权利要求31的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的肿瘤选自结肠癌、直肠癌、头颈部癌、肺癌、卵巢癌、宫颈癌、膀胱癌、食管癌、乳腺癌、肾癌、前列腺癌、胃癌、胰腺癌和脑胶质瘤。

33. 权利要求30或32的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的肿瘤为KRAS基因突变的肿瘤，例如为KRAS基因突变的结肠癌、直肠癌、肺癌或胰腺癌。

34. 权利要求30或32的抗体药物偶联物、其药学上可接受的盐、溶剂合物或所述盐的溶剂合物，其中所述的肿瘤为BRAF基因突变的肿瘤，例如选自BRAF基因突变的结肠癌、直肠癌和肺癌。

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Page 329 of 459

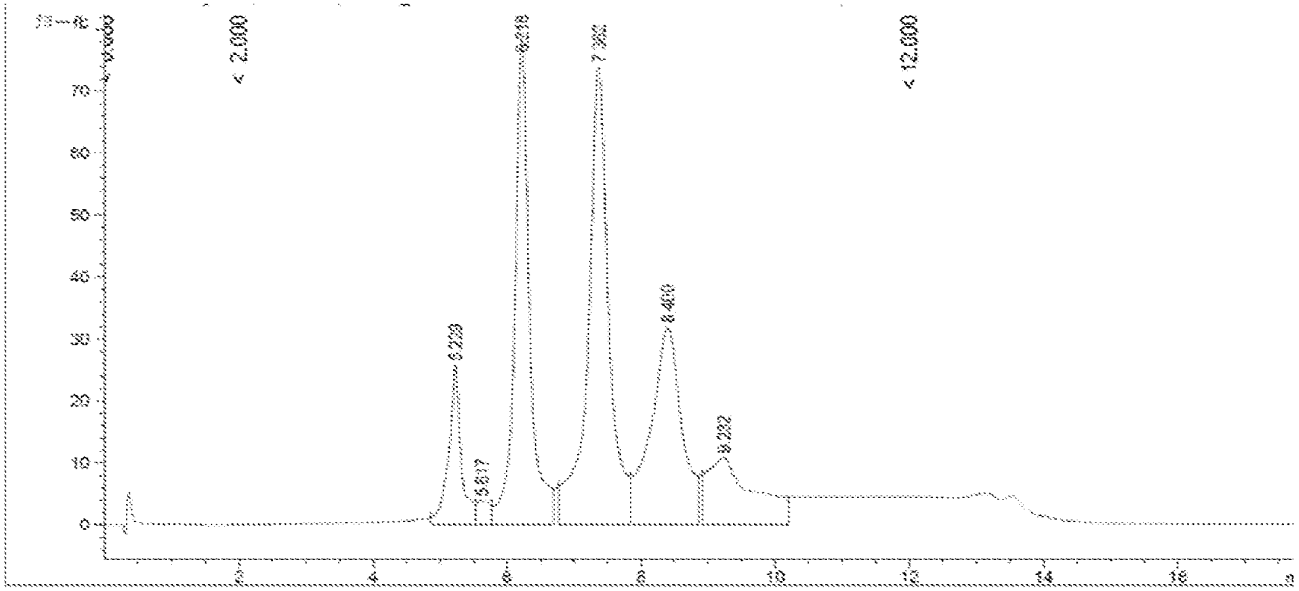

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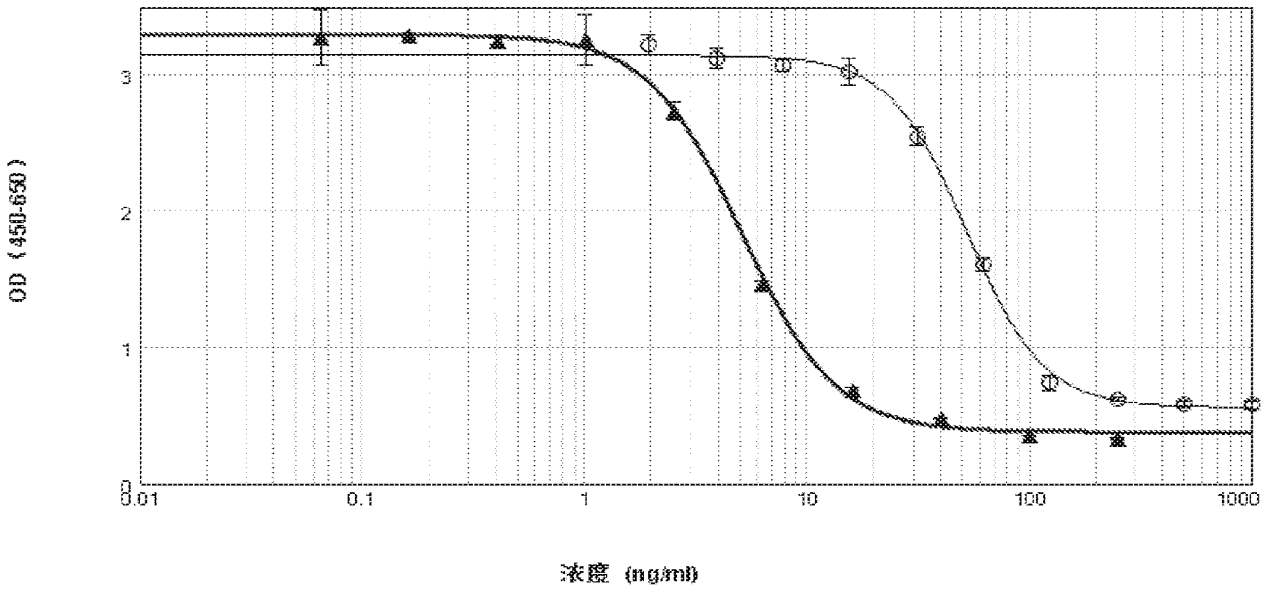

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1000

图 2

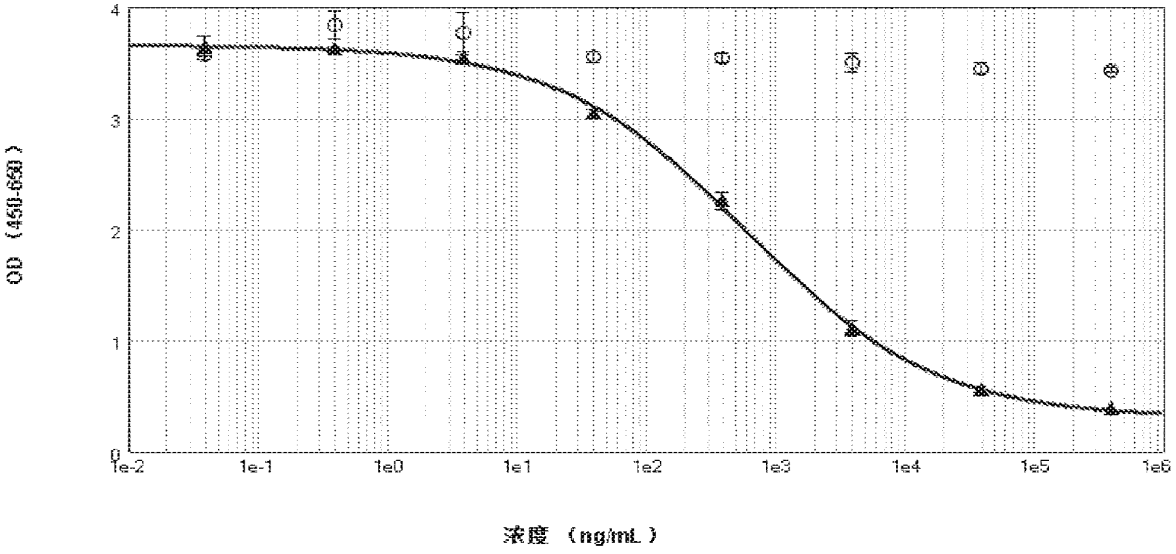


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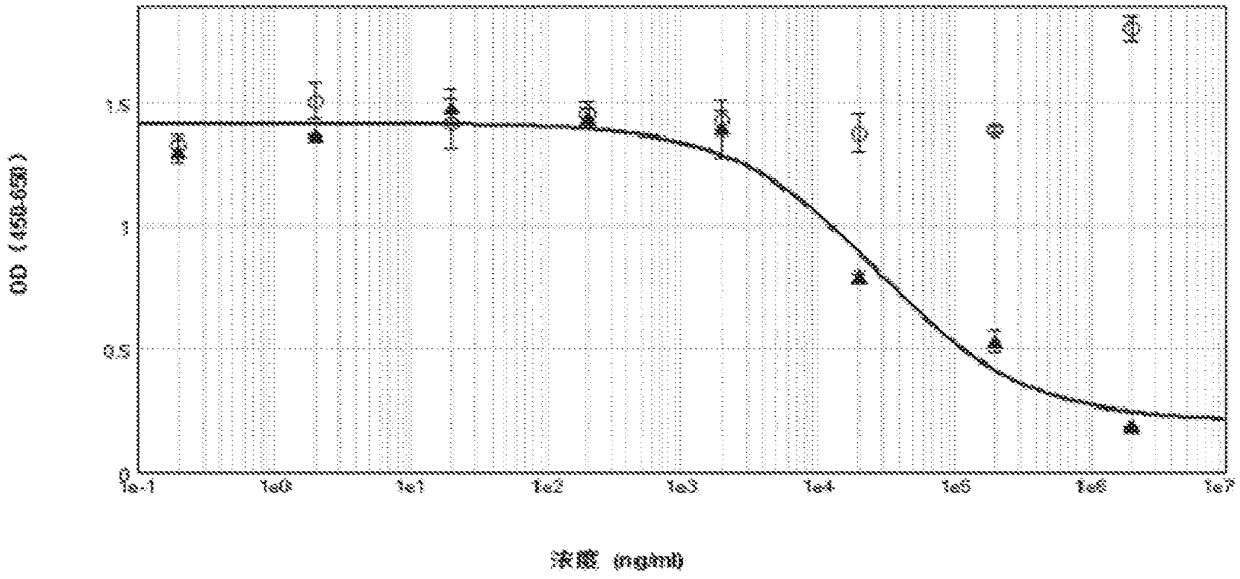


图4

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Page 331 of 459

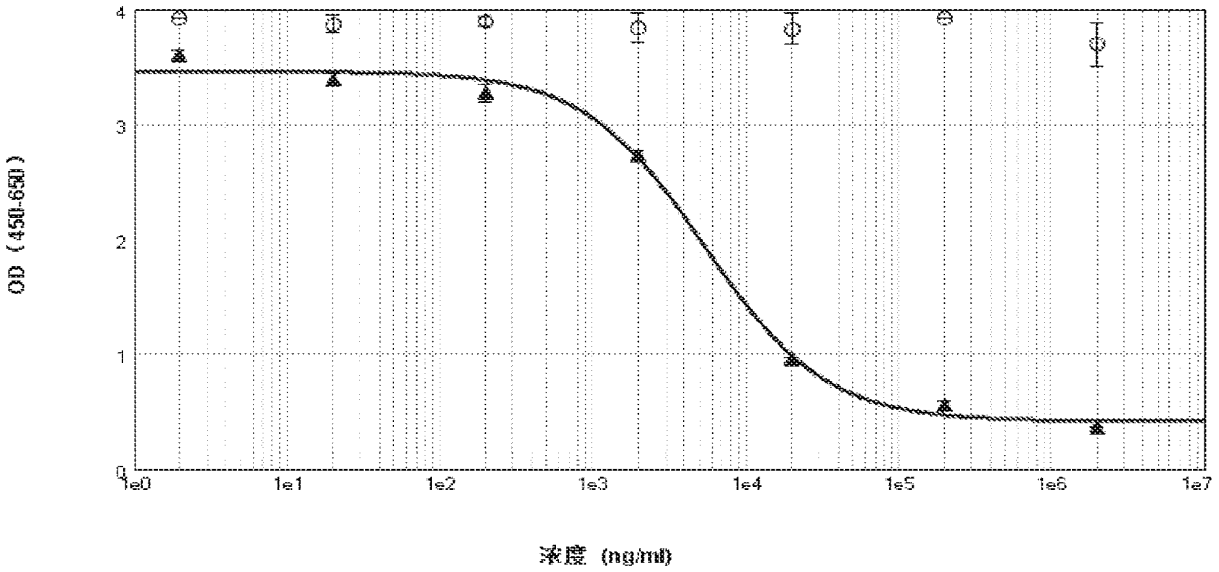

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浓度（ng/ml）

图5

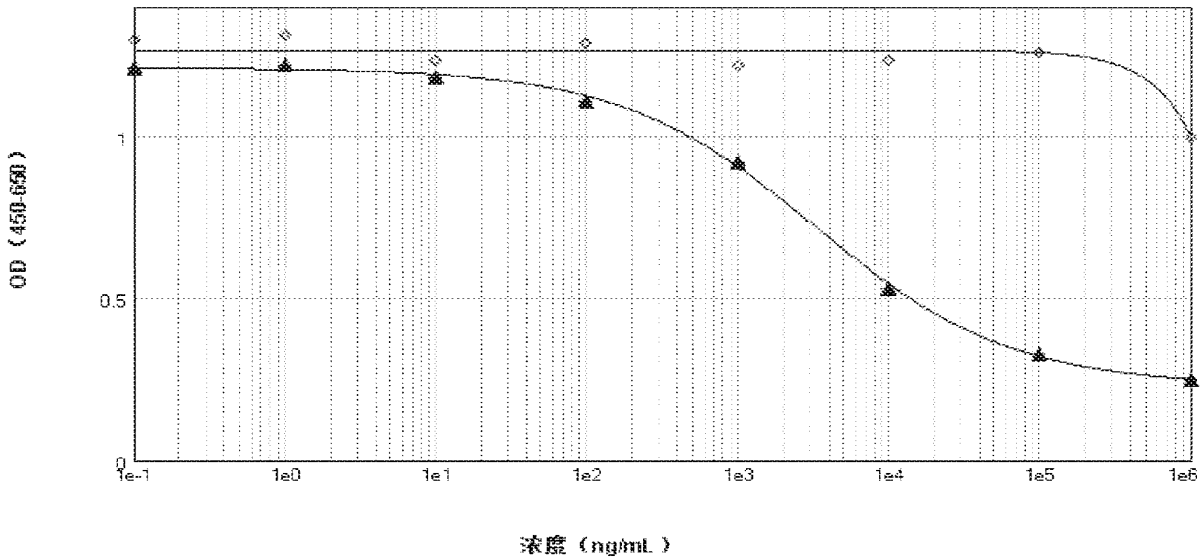

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浓度（ng/mL）

图6

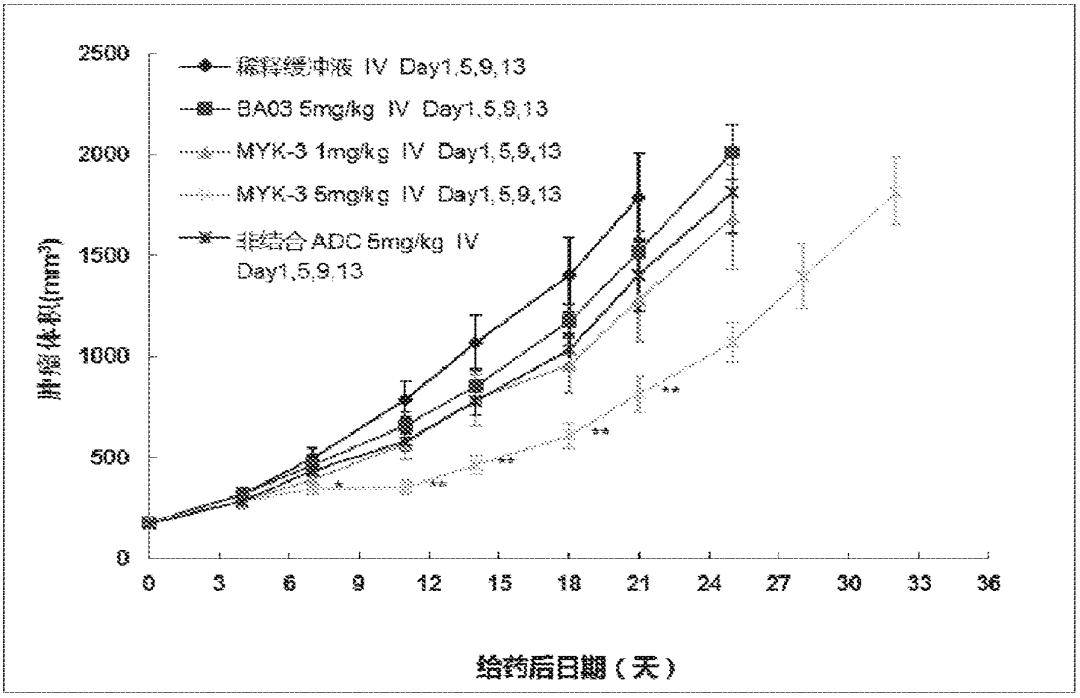


图 7

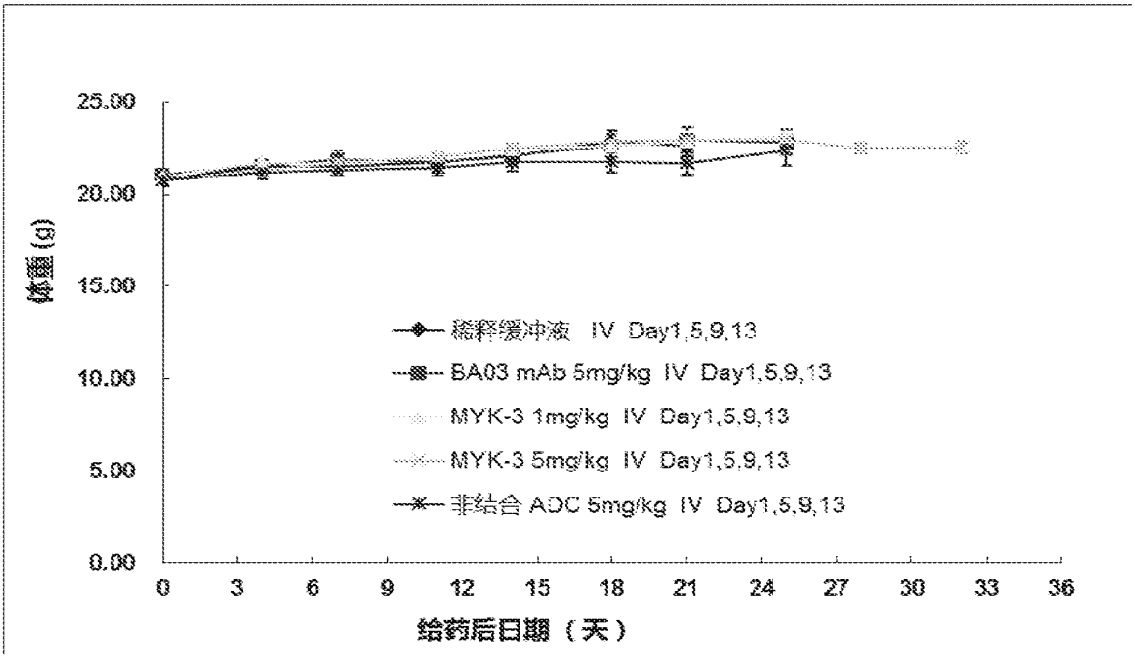


图 8

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Page 333 of 459

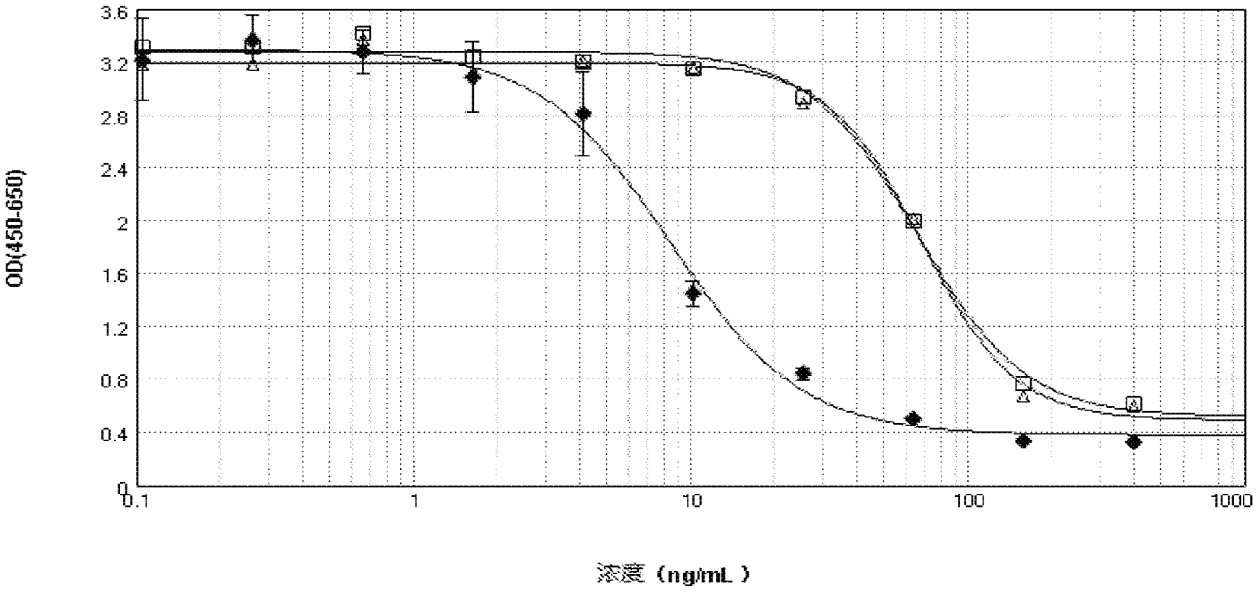


图9

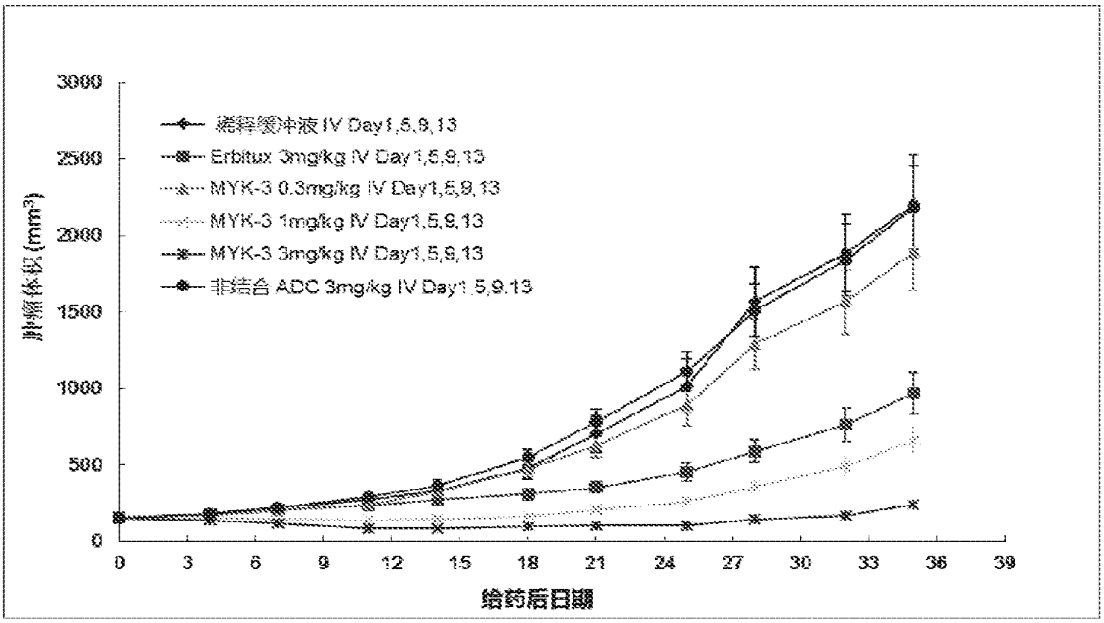


图10

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Page 334 of 459

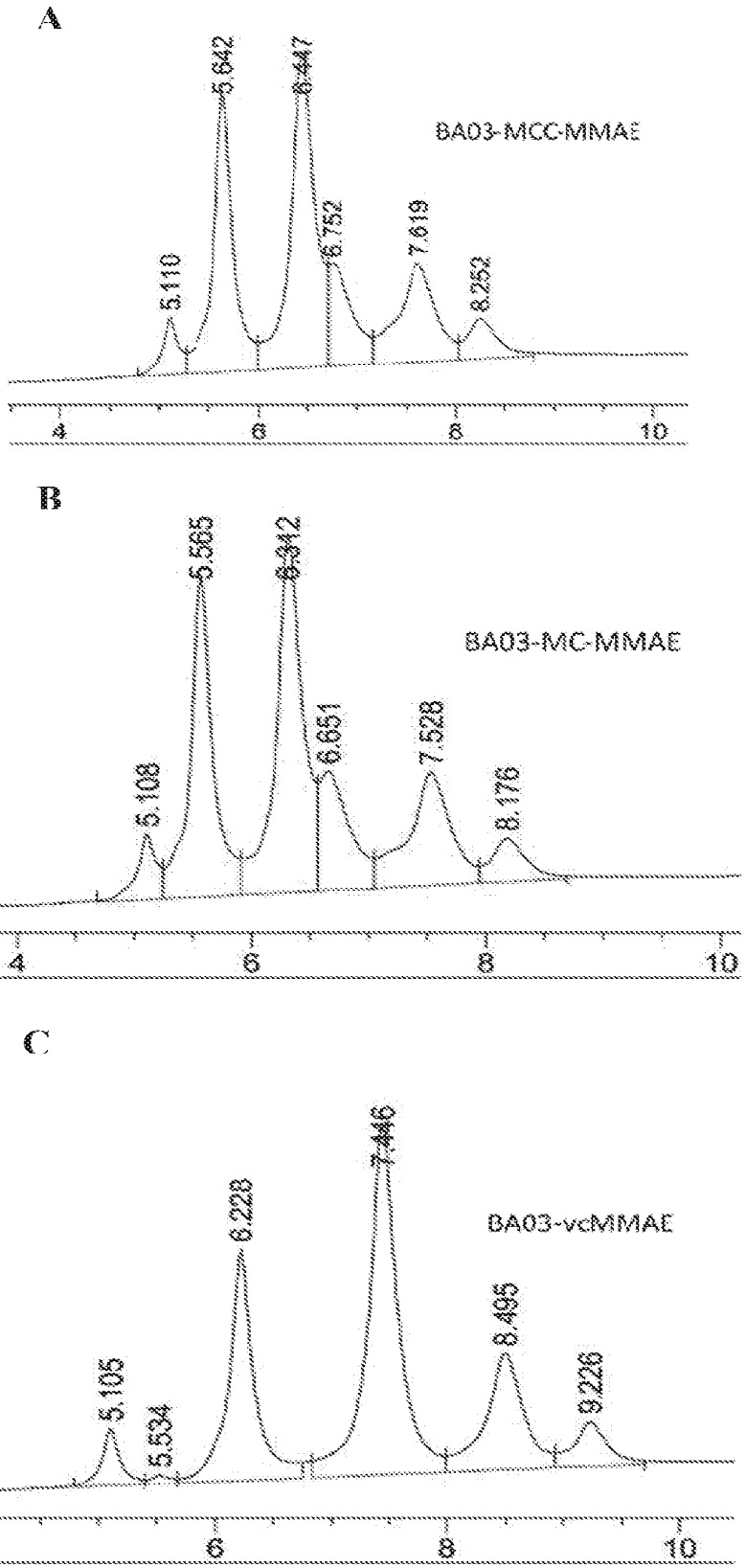

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图 11

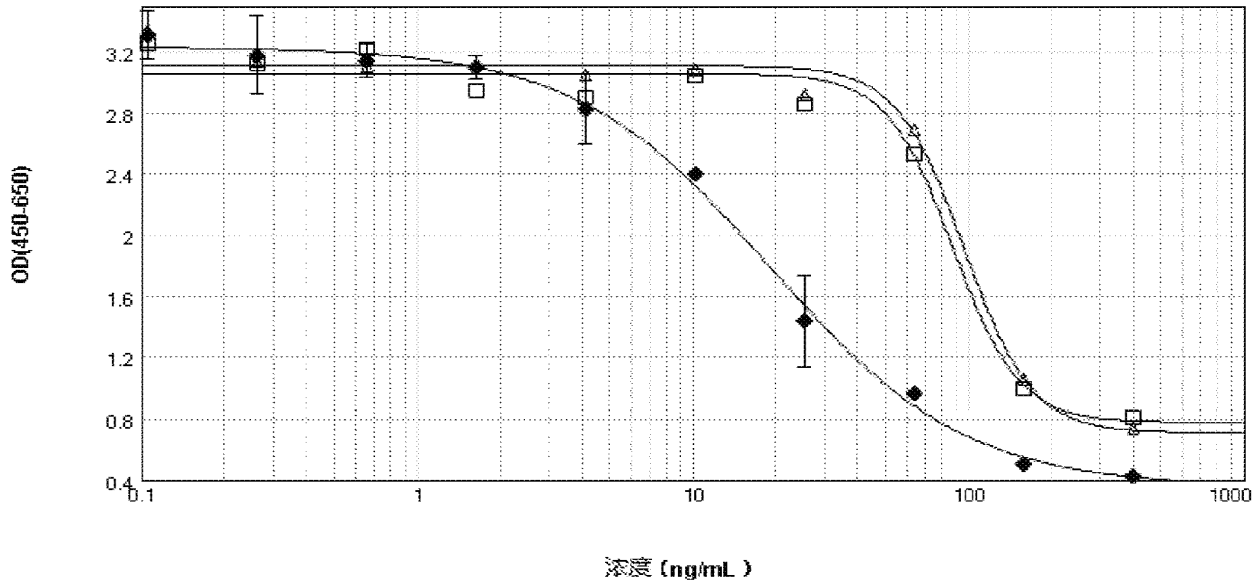


图 12

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2016/073844

A. CLASSIFICATION OF SUBJECT MATTER

A61K 47/48 (2006.01) i; A61K 45/00 (2006.01) i; A61P 35/00 (2006.01) i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K, A61P

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)

CPRSABS; CNKI; CNTXT and Search Terms: epidermal growth factor receptor, antibody, cytotoxic drug, antibody-drug-conjugate, valine, citrulline etc.

DWPI; SIPOABS; EPTXT; USTXT; WOTXT; JPTXT; ELSEVIER; CA and Search Terms: epidermal growth factor receptor, EGFR, HER, antibody, cytotoxic drug, MMAE, antibody-drug-conjugate, ADC, valine, citrulline etc.

NATIONAL BIO-SEQUENCE DATABASE OF CHINESE PATENT; Genbank; EMBL; STN and Sequences Searched: SEQ ID NOs: 1-18

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014152199 A1 (ABBVIE INC.), 25 September 2014 (25.09.2014), see abstract, claims 1-50, description, page 25, line 1 to page 36, line 3, and figure 1	1, 5-22 and 29-34
Y	WO 2014152199 A1 (ABBVIE INC.), 25 September 2014 (25.09.2014), see abstract, claims 1-50, description, page 25, line 1 to page 36, line 3, and figure 1	2-22 and 29-34
Y	CN 103772504 A (SHANGHAI JMT BIOTECHNOLOGY CO., LTD.), 07 May 2014 (07.05.2014), see claims 1-13, and embodiments 1-10	2-22 and 29-34

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 May 2016 (12.05.2016)	**18 May 2016 (18.05.2016)**
Name and mailing address of the ISA/CN: State Intellectual Property Office of the P. R. China No. 6, Xitucheng Road, Jimenqiao Haidian District, Beijing 100088, China Facsimile No.: (86-10) 62019451	Authorized officer **HE, Rui** Telephone No.: (86-10) **62411106**

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2016/073844

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 23-28
because they relate to subject matter not required to be searched by this Authority, namely:
[1] PCT Rule 39.1(iv) - a surgical method or a treatment method for the disposal of a human body or animal body.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2016/073844

Patent Documents referred in the Report	Publication Date	Patent Family	Publication Date
WO 2014152199 A1	25 September 2014	KR 20150132864 A	26 November 2015
		AU 2014240012 A1	24 September 2015
		IL 241004 D0	30 November 2015
		SG 11201507432X A	29 October 2015
		CN 105209076 A	30 December 2015
		EP 2968589 A1	20 January 2016
		CA 2906022 A1	25 September 2014
		US 2014286968 A1	25 September 2014
		TW 201519904 A	01 June 2015
CN 103772504 A	07 May 2014	None	

国际检索报告

国际申请号
PCT/CN2016/073844

A. 主题的分类

A61K 47/48(2006.01)i；A61K 45/00(2006.01)i；A61P 35/00(2006.01)i

按照国际专利分类(IPC)或者同时按照国家分类和IPC两种分类

B. 检索领域

检索的最低限度文献(标明分类系统和分类号)

A61K，A61P

包含在检索领域中的除最低限度文献以外的检索文献

在国际检索时查阅的电子数据库(数据库的名称，和使用的检索词（如使用）)

CPRSABS；CNKI；CNTXT和检索词：表皮生长因子受体，抗体，细胞毒剂药物，抗体药物缀合物，缬氨酸，瓜氨酸等 DWPI；SIPOABS；EPTXT；USTXT；WOTXT；JPTXT；ELSEVIER；CA和检索词：epidermal growth factor receptor，EGFR，HER，antibody，cytotoxic drug，MMAE，antibody-drug-conjugate，ADC，valine，citrulline等 中国专利生物序列检索系统；Genbank；EMBL；STN和检索的序列：SEQ ID NOs：1-18

C. 相关文件

类 型*	引用文件，必要时，指明相关段落	相关的权利要求
X	WO 2014152199 A1 (ABBVIE INC) 2014年 9月 25日 (2014-09-25) 参见摘要，权利要求1-50，说明书第25页第1行至第36页第3行，图1	1、5-22和29-34
Y	WO 2014152199 A1 (ABBVIE INC) 2014年 9月 25日 (2014-09-25) 参见摘要，权利要求1-50，说明书第25页第1行至第36页第3行，图1	2-22和29-34
Y	CN 103772504 A (上海津曼特生物科技有限公司) 2014年 5月 7日 (2014-05-07) 参见权利要求1-13，实施例1-10	2-22和29-34

☐ 其余文件在C栏的续页中列出。

☑ 见同族专利附件。

* 引用文件的具体类型：

“A” 认为不特别相关的表示了现有技术一般状态的文件

“E” 在国际申请日的当天或之后公布的在先申请或专利

“L” 可能对优先权要求构成怀疑的文件，或为确定另一篇引用文件的公布日而引用的或者因其他特殊理由而引用的文件（如具体说明的）

“O” 涉及口头公开、使用、展览或其他方式公开的文件

“P” 公布日先于国际申请日但迟于所要求的优先权日的文件

“T” 在申请日或优先权日之后公布，与申请不相抵触，但为了理解发明之理论或原理的在后文件

“X” 特别相关的文件，单独考虑该文件，认定要求保护的发明不是新颖的或不具有创造性

“Y” 特别相关的文件，当该文件与另一篇或者多篇该类文件结合并且这种结合对于本领域技术人员为显而易见时，要求保护的发明不具有创造性

“&” 同族专利的文件

国际检索实际完成的日期	国际检索报告邮寄日期
2016年 5月 12日	2016年 5月 18日

ISA/CN的名称和邮寄地址	受权官员
中华人民共和国国家知识产权局(ISA/CN) 中国北京市海淀区蓟门桥西土城路6号 100088 传真号 (86-10)62019451	何睿 电话号码 (86-10)62411106

表 PCT/ISA/210 (第2页) (2009年7月)

第II栏　　某些权利要求被认为是不能检索的意见(续第1页第2项)

根据条约第17条(2)(a)，对某些权利要求未做国际检索报告的理由如下:

1. ☑ 权利要求： 23-28
因为它们涉及不要求本单位进行检索的主题，即：

[1] PCT细则39.1（iv）——处置人体或者动物体的外科手术方法或治疗方法，以及诊断方法。

2. ☐ 权利要求：
因为它们涉及国际申请中不符合规定的要求的部分，以致不能进行任何有意义的国际检索， 具体地说：

3. ☐ 权利要求：
因为它们是从属权利要求，并且没有按照细则6.4(a)第2句和第3句的要求撰写。

CSPC Exhibit 1003

国际检索报告
关于同族专利的信息

国际申请号
PCT/CN2016/073844

检索报告引用的专利文件			公布日 (年/月/日)	同族专利			公布日 (年/月/日)
WO	2014152199	A1	2014年 9月 25日	KR	20150132864	A	2015年 11月 26日
				AU	2014240012	A1	2015年 9月 24日
				IL	241004	D0	2015年 11月 30日
				SG	11201507432X	A	2015年 10月 29日
				CN	105209076	A	2015年 12月 30日
				EP	2968589	A1	2016年 1月 20日
				CA	2906022	A1	2014年 9月 25日
				US	2014286968	A1	2014年 9月 25日
				TW	201519904	A	2015年 6月 1日
CN	103772504	A	2014年 5月 7日		无		

CSPC Exhibit 1003

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217-9197 (toll free).
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Asp Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Leu
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Gly Val Ile Trp Ser Gly Gly Asn Thr Asp Tyr Asn Thr Pro Phe Thr
    50                  55                  60


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


Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
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<220>
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Glu Lys Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Gly Thr Asn
            20   25   30

Ile His Trp Tyr Gln Gln Lys Pro Asp Gln Ser Pro Lys Leu Leu Ile
        35   40   45

Lys Tyr Ala Ser Glu Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly
    50   55   60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Asn Ser Leu Glu Ala
65   70   75   80

Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Asn Asn Glu Trp Pro Thr
                85   90   95

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

<210> 3
<211> 330
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<220>
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Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1   5   10   15

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Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65                  70                  75                  80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
            100                 105                 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        115                 120                 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    130                 135                 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145                 150                 155                 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                165                 170                 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            180                 185                 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        195                 200                 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    210                 215                 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
225                 230                 235                 240
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Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                245                 250                 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            260                 265                 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
        275                 280                 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
    290                 295                 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305                 310                 315                 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
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1               5                   10                  15

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

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
        35                  40                  45

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
    50                  55                  60

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
65                  70                  75                  80

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
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<220>
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<400> 8

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1               5                   10                  15


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


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1               5                   10


<210>  10
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<220>
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<400>  10

Arg Leu Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu Lys
1               5                   10                  15


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


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<400>  11

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1               5                   10


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1 5

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1 5 10

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Glu Ile Val Leu Thr Gln Ser Pro Asp Phe Gln Ser Val Thr Pro Lys
1 5 10 15

Glu Lys Val Thr Ile Thr Cys
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<210> 16
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<400> 16

Trp Tyr Gln Gln Lys Pro Asp Gln Ser Pro Lys Leu Leu Ile Lys
1 5 10 15

<210> 17
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<400> 17

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<400> 18

Gly Gln Gly Thr Lys Leu Glu Ile Lys
1 5

Doc Code: PA..
Document Description: Power of Attorney

PTO/AIA/82A (07-13)
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Application Number	15/550,995
Filing Date	August 14, 2017
First Named Inventor	Hu, Chaohong
Title	Antibody-Drug Conjugate
Art Unit	To be assigned
Examiner Name	To be assigned
Attorney Docket Number	CCPIT-002

SIGNATURE of Applicant or Patent Practitioner

Signature	/James S. Keddie/	Date (Optional)	October 3, 2017
Name	James S. Keddie	Registration Number	48920
Title (if Applicant is a juristic entity)			
Applicant Name (if Applicant is a juristic entity)			

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. If more than one applicant, use multiple forms.

☑ *Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Doc Code: PA..
Document Description: Power of Attorney

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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in either the attached transmittal letter or the boxes below.

Application Number	Filing Date

(Note: The boxes above may be left blank if information is provided on form PTO/AIA/82A.)

☑ I hereby appoint the Patent Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above: 93726

OR

☐ I hereby appoint Practitioner(s) named in the attached list (form PTO/AIA/82C) as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the patent application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above. (Note: Complete form PTO/AIA/82C.)

Please recognize or change the correspondence address for the application identified in the attached transmittal letter or the boxes above to:

☑ The address associated with the above-mentioned Customer Number

OR

☐ The address associated with Customer Number:

OR

Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the Applicant (if the Applicant is a juristic entity, list the Applicant name in the box):

SHANGHAI MIRACOGEN INC

☐ Inventor or Joint Inventor (title not required below)

☐ Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below)

☑ Assignee or Person to Whom the Inventor is Under an Obligation to Assign (provide signer's title if applicant is a juristic entity)

☐ Person Who Otherwise Shows Sufficient Proprietary Interest (e.g., a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document) (provide signer's title if applicant is a juristic entity)

SIGNATURE of Applicant for Patent

The undersigned (whose title is supplied below) is authorized to act on behalf of the applicant (e.g., where the applicant is a juristic entity).

Signature	Chaohong Hu	Date (Optional)	Aug 30, 2017
Name	CHAOHONG HU		
Title	CEO		

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. If more than one applicant, use multiple forms.

☑ Total of one (1) forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Electronic Acknowledgement Receipt

EFS ID:	30554213
Application Number:	15550995
International Application Number:	
Confirmation Number:	1813
Title of Invention:	Antibody-Drug Conjugate
First Named Inventor/Applicant Name:	Chaohong Hu
Customer Number:	93726
Filer:	James Samuel Keddie/Sherry Long
Filer Authorized By:	James Samuel Keddie
Attorney Docket Number:	CCPIT-002
Receipt Date:	03-OCT-2017
Filing Date:	
Time Stamp:	16:43:20
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Power of Attorney	CCPIT-002_POA.pdf	835656 081cc44b70484fd338ebd49f115945a6cfc9e32c	no	2

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The page size in the PDF is too large. The pages should be 8.5 x 11 or A4. If this PDF is submitted, the pages will be resized upon entry into the Image File Wrapper and may affect subsequent processing	
Information:	
Total Files Size (in bytes):	835656

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
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www.uspto.gov

U.S. APPLICATION NUMBER NO.	FIRST NAMED INVENTOR	ATTY. DOCKET NO.
15/550,995	Chaohong Hu	CCPIT-002

93726
EPA - Bozicevic Field & Francis LLP
Bozicevic, Field & Francis
201 REDWOOD SHORES PARKWAY
SUITE 200
REDWOOD CITY, CA 94065

INTERNATIONAL APPLICATION NO.	
PCT/CN2016/073844	
I.A. FILING DATE	PRIORITY DATE
02/16/2016	02/17/2015

CONFIRMATION NO. 1813
371 ACCEPTANCE LETTER

OC000000095092527

Date Mailed: 11/01/2017

NOTICE OF ACCEPTANCE OF APPLICATION UNDER 35 U.S.C 371 AND 37 CFR 1.495

The applicant is hereby advised that the United States Patent and Trademark Office, in its capacity as a Designated / Elected Office (37 CFR 1.495), has ACCEPTED the above identified international application for national patentability examination in the United States Patent and Trademark Office.

The United States Application Number assigned to the application is shown above. A Filing Receipt will be issued for the present application in due course. **THE DATE APPEARING ON THE FILING RECEIPT AS THE "FILING DATE or 371(c) DATE" IS THE DATE ON WHICH THE LAST OF THE 35 U.S.C. 371 (c)(1) and (c)(2) REQUIREMENTS HAS BEEN RECEIVED IN THE OFFICE. THIS DATE IS SHOWN BELOW.** The filing date of the above identified application is the international filing date of the international application (Article 11(3) and 35 U.S.C. 363)

08/14/2017
DATE OF RECEIPT OF 35 U.S.C.
371(c)(1) and (c)(2) REQUIREMENTS

The following items have been received:

- Indication of Small Entity Status
- Copy of the International Application filed on 08/14/2017
- English Translation of the IA filed on 08/14/2017
- Copy of the International Search Report filed on 08/14/2017
- Preliminary Amendments filed on 08/14/2017
- Information Disclosure Statements filed on 08/14/2017
- Sequence Listing txt file (EFS or physical medium) filed on 08/14/2017
- Inventor's Oath or Declaration filed on 08/14/2017
- Sequence Listing (PDF or paper) filed on 08/14/2017
- Request for Immediate Examination filed on 08/14/2017
- U.S. Basic National Fees filed on 08/14/2017
- Authorize Access to Search Results filed on 08/14/2017
- Priority Documents filed on 08/14/2017
- Power of Attorney filed on 10/03/2017
- Authorization to Permit Access filed on 08/14/2017
- Application Data Sheet (37 CFR 1.76) filed on 08/14/2017

Applicant is reminded that any communications to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above (37 CFR 1.5)

INDIA L EVANS

Telephone: (571) 272-9085

MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET

Substitute for Form PTO-1360
(For use with Form PTO/SB/06)

Application Number	Filing Date
15550995	

Applicant(s) Chaohong Hu

* May be used for additional claims or amendments

CLAIMS	AS FILED		AFTER FIRST AMENDMENT		AFTER SECOND AMENDMENT			*		*		*	
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Total Claims	40		23		0								

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
15/550,995	08/14/2017		860	CCPIT-002	23	1

CONFIRMATION NO. 1813

FILING RECEIPT

93726
EPA - Bozicevic Field & Francis LLP
Bozicevic, Field & Francis
201 REDWOOD SHORES PARKWAY
SUITE 200
REDWOOD CITY, CA 94065



Date Mailed: 11/01/2017

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Chaohong Hu, SHANGHAI, CHINA;

Applicant(s)

SHANGHAI MIRACOGEN INC, SHANGHAI, CHINA

Power of Attorney: None

Domestic Priority data as claimed by applicant

This application is a 371 of PCT/CN2016/073844 02/16/2016

Foreign Applications (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.)
CHINA 201510085038.8 02/17/2015

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 10/28/2017
The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 15/550,995**
Projected Publication Date: 02/08/2018
Non-Publication Request: No

Early Publication Request: No
**** SMALL ENTITY ****
Title

Antibody-Drug Conjugate

Preliminary Class

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

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Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

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For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

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This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

SelectUSA

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 15/550,995

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	140		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	240		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	360		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	23 minus 20 =	* 3	x 40 =	120	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	1 minus 3 =	*	x 210 =	0.00			
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			0.00			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				0.00			
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	860		TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)		RATE($)	ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

To: docket@bozpat.com,,
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 93726

Nov 01, 2017 03:48:13 AM

Dear PAIR Customer:

EPA - Bozicevic Field & Francis LLP
Bozicevic, Field & Francis
201 REDWOOD SHORES PARKWAY
SUITE 200
REDWOOD CITY, CA 94065
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 93726 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

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Application	Document	Mailroom Date	Attorney Docket No.
15550995	M903	11/01/2017	CCPIT-002
	APP.FILE.REC	11/01/2017	CCPIT-002

To view your correspondence online or update your email addresses, please visit us anytime at https://sportal.uspto.gov/secure/myportal/privatepair.

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Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

PATENT COOPERATION TREATY

TRANSLATION

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	18/05/2016
Applicant's or agent's file reference IEC150092PCT	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CN2016/073844	16.02.2016	17.02.2015

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

A61K47/48(2006.01)i;A61K45/00(2006.01)i;A61P35/00(2006.01):

Applicant

SHANGHAI MIRACOGEN INC

1. This opinion contains indications relating to the following items:

- ☒ Box No. I — Basis of the opinion
- ☐ Box No. II — Priority
- ☒ Box No. III — Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV — Lack of unity of invention
- ☒ Box No. V — Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI — Certain documents cited
- ☒ Box No. VII — Certain defects in the international application
- ☒ Box No. VIII — Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/CN	Date of completion of this opinion	Authorized officer
Facsimile No.		Telephone No.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/CN2016/073844

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into _____ , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☒ in electronic form

   b. (time)

      ☒ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ 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 in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/CN2016/073844

Box No. III **Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application

☒ claims Nos. See the following claims

because:

☒ the said international application, or the said claims Nos. 23-38 relate to the following subject matter which does not require an international search (*specify*):

[1] PCT Rule 39.1(iv) -- methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _____ are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):

☒ no international search report has been established for said claims Nos. 23-38

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/CN2016/073844

Box No. V **Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	2-4, 21, 22, 33 and 34	YES
	Claims	1, 5-20 and 29-32	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-22 and 29-34	NO
Industrial applicability (IA)	Claims	1-22 and 29-34	YES
	Claims	None	NO

2. Citations and explanations:

[1] The written opinion by the International Search Authority with regard to novelty, inventive step or industrial applicability is established on the basis of the subject matter that could be reasonably expected to be claimed. See Box No. VIII for details.

[2] The following reference documents are cited:

[3] D1: WO 2014152199 A1, 25 September 2014 (25.09.2014)

[4] D2: CN 103772504 A, 07 May 2014 (07.05.2014)

[5] Novelty and Inventive Step (PCT Article 33(2) and PCT Article 33(3))

[6] D1 (see abstract, claims 1-50, description, line 1 on page 25 to line 3 on page 36, and Fig. 1) discloses an antibody-drug conjugate, wherein the

antibody is an anti-EGFR antibody and the drug can be NMAE, and also discloses a composition comprising the antibody-drug conjugate and a use of the antibody-drug conjugate.

[7] D2 (see claims 1-13, and Examples 1-10) discloses an anti-EGFR antibody BA03, wherein the amino acid sequences of the variable region and the constant region thereof are the same as the antibody of the present application.

[8] The drug in the conjugate disclosed in D1 is a cytotoxic agent, and the drug is covalently connected to the anti-EGFR antibody via a linker. Therefore, claim 1 of the present application lacks novelty.

[9] D2 discloses an anti-EGFR antibody, wherein the sequence of the variable region thereof is the same as the sequence defined in claims 2-4 of the present application. Hence, it would be obvious for a person skilled in the art to arrive at the technical solutions of claims 2-4 by combining D1 and D2. Therefore, claims 2-4 do not involve an inventive step.

[10] The heavy chain constant region of the anti-EGFR antibody disclosed in D1 differs from SEQ ID NO: 3 in respect of three amino acids, and the light chain constant region of the anti-EGFR antibody disclosed in D1 differs from SEQ ID NO: 4 in respect of one amino acid. Therefore, given that

the claims to which claims 5-9 refer lack novelty/do not involve an inventive step, claims 5-9 lack novelty/do not involve an inventive step either.

[11] The structure of the antibody-drug conjugate disclosed in D1 falls within the scope of formula I of claim 10 of the present application. Therefore, given that the claims to which claim 10 refers lack novelty/do not involve an inventive step, claim 10 lacks novelty/does not involve an inventive step either.

[12] D1 discloses that the drug can be NMAE, MMAF and the like. Therefore, given that the claims to which claims 11 and 12 of the present application refer lack novelty/do not involve an inventive step, claims 11 and 12 lack novelty/do not involve an inventive step either.

[13] D1 discloses that the linker can be a cleavable linker and can be MC, PAB, SMCC, SPP, valine-citrulline and the like. Therefore, given that the claims to which claims 13 and 14 of the present application refer lack novelty/do not involve an inventive step, claims 13 and 14 lack novelty/do not involve an inventive step either.

[14] D1 discloses a specific manner in which MMAE is connected to the antibody. The corresponding structure is the same as that defined in claim 15 of the present application, and the number of the

drug can be 2 or 4. Therefore, given that the claims to which claim 15 refers lack novelty/do not involve an inventive step, claim 15 lacks novelty/does not involve an inventive step either.

[15] D1 discloses a composition comprising the antibody-drug conjugate, and discloses that the antibody-drug conjugate can be used for diagnosing and treating an EGFR-related disease, and can be used for diagnosing and treating a cancer, such as lung cancer, colon cancer, head and neck cancer, breast cancer, and the like. Therefore, given that the claims to which claims 16-20 and 29-32 of the present application refer lack novelty/do not involve an inventive step, claims 16-20 and 29-32 lack novelty/do not involve an inventive step either.

[16] In light of D1, a person skilled in the art could expect that the anti-EGFR antibody-drug conjugate can be used for treating a tumour associated with KRAS gene mutation and a tumour associated with BRAF gene mutation. Therefore, claims 21, 22, 33 and 34 of the present application do not involve an inventive step.

[17] Industrial Applicability (PCT Article 33(4))

[18] The invention according to claims 1-22 and 29-34 of the present application can be made and used in the technical field of biopharmaceuticals and is therefore industrially applicable.

Box No. VII **Certain defects in the international application**

The following defects in the form or contents of the international application have been noted:

[1] Multiple dependent claims 8, 10, 11, 15, 29 and 31 refer to preceding multiple dependent claims. Therefore, these claims do not comply with PCT Rule 6.4(a).

Box No. VIII **Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

[1] The expression "mutant" is used in claims 2-5 and 8. A person skilled in the art could not ascertain the scope of the expression. Therefore, these claims are unclear and do not comply with PCT Article 6.

[2] For convenience in conducting the search and establishing the written opinion, the technical solutions of claims 2-5 and 8 are contemplated to be technical solutions with the expression "mutant" having been deleted.

[3] The expression "and the like" is used in claim 12. This results in claims 12 being unclear and not complying with PCT Article 6.

[4] For convenience in conducting the search and establishing the written opinion, the technical solution of claim 12 is contemplated to be a technical solution with the expression "and the like" having been deleted.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference IEC150092PCT	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/CN2016/073844	International filing date *(day/month/year)* 16 February 2016 (16.02.2016)	Priority date *(day/month/year)* 17 February 2015 (17.02.2015)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant SHANGHAI MIRACOGEN INC		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☒	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 22 August 2017 (22.08.2017)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Lingfei Bai e-mail: pct.team2@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

TRANSLATION

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	18/05/2016
Applicant's or agent's file reference IEC150092PCT	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CN2016/073844	16.02.2016	17.02.2015

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

A61K47/48(2006.01)i;A61K45/00(2006.01)i;A61P35/00(2006.01):

Applicant

SHANGHAI MIRACOGEN INC

1. This opinion contains indications relating to the following items:

- ☒ Box No. I — Basis of the opinion
- ☐ Box No. II — Priority
- ☒ Box No. III — Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV — Lack of unity of invention
- ☒ Box No. V — Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI — Certain documents cited
- ☒ Box No. VII — Certain defects in the international application
- ☒ Box No. VIII — Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/CN	Date of completion of this opinion	Authorized officer
Facsimile No.		Telephone No.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/CN2016/073844

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into _____ , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☒ in electronic form

   b. (time)

      ☒ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ 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 in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/CN2016/073844

Box No. III **Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application

☒ claims Nos. See the following claims

because:

☒ the said international application, or the said claims Nos. 23-38 relate to the following subject matter which does not require an international search (*specify*):

[1] PCT Rule 39.1(iv) -- methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _____ are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):

☒ no international search report has been established for said claims Nos. 23-38

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/CN2016/073844

Box No. V **Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	2-4, 21, 22, 33 and 34	YES
	Claims	1, 5-20 and 29-32	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-22 and 29-34	NO
Industrial applicability (IA)	Claims	1-22 and 29-34	YES
	Claims	None	NO

2. Citations and explanations:

[1] The written opinion by the International Search Authority with regard to novelty, inventive step or industrial applicability is established on the basis of the subject matter that could be reasonably expected to be claimed. See Box No. VIII for details.

[2] The following reference documents are cited:

[3] D1: WO 2014152199 A1, 25 September 2014 (25.09.2014)

[4] D2: CN 103772504 A, 07 May 2014 (07.05.2014)

[5] Novelty and Inventive Step (PCT Article 33(2) and PCT Article 33(3))

[6] D1 (see abstract, claims 1-50, description, line 1 on page 25 to line 3 on page 36, and Fig. 1) discloses an antibody-drug conjugate, wherein the

antibody is an anti-EGFR antibody and the drug can be NMAE, and also discloses a composition comprising the antibody-drug conjugate and a use of the antibody-drug conjugate.

[7] D2 (see claims 1-13, and Examples 1-10) discloses an anti-EGFR antibody BA03, wherein the amino acid sequences of the variable region and the constant region thereof are the same as the antibody of the present application.

[8] The drug in the conjugate disclosed in D1 is a cytotoxic agent, and the drug is covalently connected to the anti-EGFR antibody via a linker. Therefore, claim 1 of the present application lacks novelty.

[9] D2 discloses an anti-EGFR antibody, wherein the sequence of the variable region thereof is the same as the sequence defined in claims 2-4 of the present application. Hence, it would be obvious for a person skilled in the art to arrive at the technical solutions of claims 2-4 by combining D1 and D2. Therefore, claims 2-4 do not involve an inventive step.

[10] The heavy chain constant region of the anti-EGFR antibody disclosed in D1 differs from SEQ ID NO: 3 in respect of three amino acids, and the light chain constant region of the anti-EGFR antibody disclosed in D1 differs from SEQ ID NO: 4 in respect of one amino acid. Therefore, given that

the claims to which claims 5-9 refer lack novelty/do not involve an inventive step, claims 5-9 lack novelty/do not involve an inventive step either.

[11] The structure of the antibody-drug conjugate disclosed in D1 falls within the scope of formula I of claim 10 of the present application. Therefore, given that the claims to which claim 10 refers lack novelty/do not involve an inventive step, claim 10 lacks novelty/does not involve an inventive step either.

[12] D1 discloses that the drug can be NMAE, MMAF and the like. Therefore, given that the claims to which claims 11 and 12 of the present application refer lack novelty/do not involve an inventive step, claims 11 and 12 lack novelty/do not involve an inventive step either.

[13] D1 discloses that the linker can be a cleavable linker and can be MC, PAB, SMCC, SPP, valine-citrulline and the like. Therefore, given that the claims to which claims 13 and 14 of the present application refer lack novelty/do not involve an inventive step, claims 13 and 14 lack novelty/do not involve an inventive step either.

[14] D1 discloses a specific manner in which MMAE is connected to the antibody. The corresponding structure is the same as that defined in claim 15 of the present application, and the number of the

drug can be 2 or 4. Therefore, given that the claims to which claim 15 refers lack novelty/do not involve an inventive step, claim 15 lacks novelty/does not involve an inventive step either.

[15] D1 discloses a composition comprising the antibody-drug conjugate, and discloses that the antibody-drug conjugate can be used for diagnosing and treating an EGFR-related disease, and can be used for diagnosing and treating a cancer, such as lung cancer, colon cancer, head and neck cancer, breast cancer, and the like. Therefore, given that the claims to which claims 16-20 and 29-32 of the present application refer lack novelty/do not involve an inventive step, claims 16-20 and 29-32 lack novelty/do not involve an inventive step either.

[16] In light of D1, a person skilled in the art could expect that the anti-EGFR antibody-drug conjugate can be used for treating a tumour associated with KRAS gene mutation and a tumour associated with BRAF gene mutation. Therefore, claims 21, 22, 33 and 34 of the present application do not involve an inventive step.

[17] Industrial Applicability (PCT Article 33(4))

[18] The invention according to claims 1-22 and 29-34 of the present application can be made and used in the technical field of biopharmaceuticals and is therefore industrially applicable.

Box No. VII **Certain defects in the international application**

The following defects in the form or contents of the international application have been noted:

[1] Multiple dependent claims 8, 10, 11, 15, 29 and 31 refer to preceding multiple dependent claims. Therefore, these claims do not comply with PCT Rule 6.4(a).

Box No. VIII **Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

[1] The expression "mutant" is used in claims 2-5 and 8. A person skilled in the art could not ascertain the scope of the expression. Therefore, these claims are unclear and do not comply with PCT Article 6.

[2] For convenience in conducting the search and establishing the written opinion, the technical solutions of claims 2-5 and 8 are contemplated to be technical solutions with the expression "mutant" having been deleted.

[3] The expression "and the like" is used in claim 12. This results in claims 12 being unclear and not complying with PCT Article 6.

[4] For convenience in conducting the search and establishing the written opinion, the technical solution of claim 12 is contemplated to be a technical solution with the expression "and the like" having been deleted.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference IEC150092PCT	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/CN2016/073844	International filing date *(day/month/year)* 16 February 2016 (16.02.2016)	Priority date *(day/month/year)* 17 February 2015 (17.02.2015)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant SHANGHAI MIRACOGEN INC		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☒	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 22 August 2017 (22.08.2017)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Lingfei Bai e-mail: pct.team2@wipo.int

Form PCT/IB/373 (January 2004)

专 利 合 作 条 约

发信人：国际检索单位

收信人：
100037
中国北京市西城区阜成门外大街2号万通新世界广场8层

中国国际贸易促进委员会专利商标事务所

PCT

国际检索单位书面意见

(PCT细则43之二 .1)

发文日 (年/月/日)	2016年 5月 18日
申请人或代理人的档案号	关于后续行为
IEC150092PCT	见下面第2段

国际申请号	国际申请日 (年/月/日)	优先权日 (年/月/日)
PCT/CN2016/073844	2016年 2月 16日	2015年 2月 17日

国际专利分类（IPC）或国家分类及IPC
A61K 47/48(2006.01)i; A61K 45/00(2006.01)i; A61P 35/00(2006.01)i

申请人
上海美雅珂生物技术有限责任公司

1. 本意见包括关于下列各项标明的内容：

- [x] 第I栏 意见的基础
- [ ] 第II栏 优先权
- [x] 第III栏 不做出关于新颖性、创造性和工业实用性的意见
- [ ] 第IV栏 缺乏发明的单一性
- [x] 第V栏 按照细则43之二.1(a)(i)关于新颖性、创造性或工业实用性的推断性声明；支持这种声明的引证和解释
- [ ] 第VI栏 某些引用的文件
- [x] 第VII栏 国际申请中的某些缺陷
- [x] 第VIII栏 对国际申请的某些意见

2. 后续行为

如果提出初步审查要求书，本次意见将被视为国际初步审查单位(IPEA)的一次书面意见，除非申请人选择的国际初步审查单位非本机构，而且所选国际初步审查单位已按照细则66.1之二(b)通知国际局将不考虑国际检索单位的书面意见时例外。

如本书面意见被视为国际初步审查单位的书面意见，则请申请人在自PCT/ISA/220表发文日起3个月或自优先权日起22个月内（以后届满者为准）向国际初步审查单位提交书面答复并提交修改（如适用）。

进一步的选择参见PCT/ISA/220表。

ISA/CN的名称和邮寄地址	完成本意见的日期	受权官员
中华人民共和国国家知识产权局 中国北京市海淀区蓟门桥西土城路6号 100088	2016年 5月 13日	何睿
传真号 （86－10）62019451		电话号码 (86-10)62411106

表 PCT/ISA/237 （扉页） (2011年7月)

国际检索单位书面意见

国际申请号
PCT/CN2016/073844

第I栏 意见的基础

1. 关于语言，本意见的制定基于：
   - [x] 国际申请提交时使用的语言。
   - [ ] 该国际申请的_____语言译文，为了国际检索的目的提供该种语言的译文(细则12.3(a)和23.1(b))。

2. [ ] 本意见的制定考虑了本单位许可或被通知的根据细则91所做出的明显错误更正（细则 43之二1（a））。

3. 关于国际申请中所公开的任何对要求保护的发明必要的核苷酸和/或氨基酸序列，本意见是在下列基础上制定的：

   a. (提交提供)
      - [ ] 纸件形式
      - [x] 电子形式

   b. (提交时间)
      - [x] 含在申请提交时的国际申请中
      - [ ] 以电子形式与国际申请一起提交
      - [ ] 为检索之用随后提交本单位

4. [ ] 另外，在提交/提供了多个版本或副本的序列表的情况下，提供了随后或附加副本中的信息与申请时提交的申请中的信息相同或未超出申请时提交的申请中的信息范围（如适用）的所需声明。

5. 补充意见：

CSPC Exhibit 1003

第III栏　　不做出关于新颖性、创造性和工业实用性的意见

对于

☐ 整个国际申请

☑ 权利要求 参见以下权利要求

没有审查要求保护的发明看来是否具备新颖性、创造性(非显而易见性)，或者工业实用性的问题，

因为：

☑ 该国际申请，或者该权利要求 23-28 涉及不要求国际检索的主题(具体说明）：

[1] PCT细则39.1（iv）——处置人体或者动物体的外科手术方法或治疗方法，以及诊断方法。

☐ 说明书、权利要求书或者附图(下面特别指明的部分)或者所述权利要求_____不清楚，以致不能够形成任何有意义的意见(具体说明)：

☐ 权利要求书或所述权利要求_____没有得到说明书的充分支持，以致不能形成任何有意义的意见（具体说明）：

☑ 对所述权利要求 23-28 没有做出国际检索报告。

☐ 没有序列表，无法做出有意义的意见；申请人没有在规定的期限内：

☐ 没有提供符合行政规程附件C中所规定的标准的纸件形式的序列表，并且国际检索单位也未获得式和方式可以被接受的序列表。

☐ 没有提供符合行政规程附件c中所规定的标准的电子形式的序列表，并且国际检索单位也未获得式和方式可以被接受的序列表。

☐ 在答复根据细则13之三.1(a)或（b)的通知提交序列表时，没有缴纳所要求的后提交费。

☐ 详情见补充栏

第V栏　　按细则43之二.1（a）（i）关于新颖性、创造性或工业实用性的推测性声明；支持这种声明的引证和解释

1. 声明

新颖性 (N)	权利要求	2-4、21、22、33和34	是
	权利要求	1、5-20和29-32	否
创造性 (IS)	权利要求	无	是
	权利要求	1-22和29-34	否
工业实用性 (IA)	权利要求	1-22和29-34	是
	权利要求	无	否

2. 引证和解释：

[1] 由国际检索单位作出的关于新颖性、创造性或工业实用性的书面意见是基于可以被合理预期要求保护的主题，详情可见第Ⅷ栏。

[2] 引用以下的对比文件

[3] D1：WO 2014152199A1　　25.9月2014（25.09.2014）

[4] D2：CN 103772504A　　07.5月2014（07.05.2014）

[5] 新颖性和创造性（PCT条约第33条第2和3款）

[6] D1（参见摘要，权利要求1-50，说明书第25页第1行至第36页第3行，图1）公开了一种抗体药物缀合物，所述抗体为抗EGFR抗体，所述药物可以为MMAE，并公开了包含所述抗体药物缀合物的组合物和所述抗体药物缀合物的用途。

[7] D2（参见权利要求1-13，实施例1-10）公开了一种抗EGFR抗体BA03，其可变区和恒定区的氨基酸序列与本申请的抗体相同。

[8] D1公开的缀合物中的药物属于细胞毒剂，药物与抗EGFR抗体通过接头共价连接。因此，权利要求1不具备新颖性。

[9] D2公开了一种抗EGFR抗体，其可变区序列与权利要求2-4中限定的序列相同。因此，在D1的基础上结合D2得到权利要求2-4的技术方案，对本领域技术人员来说是显而易见的。权利要求2-4不具备创造性。

[10] D1公开的抗EGFR抗体的重链恒定区与SEQ ID NO:3有3个氨基酸不同，轻链恒定区与SEQ ID NO:4有一个氨基酸不同。当其引用的权利要求不具备新颖性/创造性时，权利要求5-9也不具备新颖性/创造性。

[11] D1公开的抗体药物缀合物的结构落入式Ⅰ的范围内。当其引用的权利要求不具备新颖性/创造性时，权利要求10也不具备新颖性/创造性。

[12] D1公开所述药物可以为MMAE、MMAF等。当其引用的权利要求不具备新颖性/创造性时，权利要求11和12也不具备新颖性/创造性。

[13] D1公开所述接头可为可切割接头，可为MC、PAB、SMCC、SPP、缬氨酸-瓜氨酸等。当其引用的权利要求不具备新颖性/创造性时，权利要求13和14也不具备新颖性/创造性。

[14] D1公开了一种具体的MMAE连接到抗体的连接方式，其与权利要求15限定的结构相同，药物个数可以为2或4。当其引用的权利要求不具备新颖性/创造性时，权利要求15也不具备新颖性/创造性。

[15] D1公开了包含所述抗体药物缀合物的组合物，并公开了所述抗体药物缀合物可用于诊断和治疗EGFR相关疾病，可用于诊断和治疗癌症，例如肺癌、结肠癌、头颈部癌、乳腺癌等。当其引用的权利要求不具备新颖性/创造性时，权利要求16-20、29-32也不具备新颖性/创造性。

[16] 在D1的启示下，本领域技术人员能够预期所述抗EGFR抗体-药物缀合物可以用于治疗KRAS基因突变的肿瘤和BRAF基因突变的肿瘤。因此，权利要求21、22、33、34不具备创造性。

[17] 工业实用性（PCT条约第33条第4款）

[18] 权利要求1-22、29-34所述发明可在生物医药技术领域制造和使用，因此符合工业实用性的规定。

CSPC Exhibit 1003

第VII栏　　国际申请中的某些缺陷

国际申请具有下列形式或内容缺陷：

[1] 由于多项从属权利要求8、10、11、15、29、31引用了在前的多项从属权利要求，所述权利要求不满足PCT细则6.4（a）的规定。

CSPC Exhibit 1003
Page 390 of 459

第VIII栏　　对国际申请的某些意见

就权利要求、说明书和附图的清楚性或者就权利要求是否得到说明书的充分支持提出以下意见：

[1] 权利要求2-5、8中使用了“突变体”，本领域技术人员无法确定其范围。因此，所述权利要求不清楚，不符合PCT条约第6条的规定。

[2] 为了便于检索和出具书面意见，将权利要求2-5、8的技术方案预期为删除“突变体”后的技术方案。

[3] 权利要求12中使用了“等”，这导致权利要求12不清楚，不符合PCT条约第6条的规定。

[4] 为了便于检索和出具书面意见，将权利要求12的技术方案预期为删除“等”后的技术方案。

CSPC Exhibit 1003

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
15/550,995	08/14/2017	Chaohong Hu	CCPIT-002

CONFIRMATION NO. 1813

PUBLICATION NOTICE

93726
EPA - Bozicevic Field & Francis LLP
Bozicevic, Field & Francis
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SUITE 200
REDWOOD CITY, CA 94065

OC00000097277784

Title: Antibody-Drug Conjugate

Publication No.US-2018-0036423-A1
Publication Date:02/08/2018

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently http://www.uspto.gov/patft/.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Public Records Division. The Public Records Division can be reached by telephone at (571) 272-3150 or (800) 972-6382, by facsimile at (571) 273-3250, by mail addressed to the United States Patent and Trademark Office, Public Records Division, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently https://portal.uspto.gov/pair/PublicPair. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Office of Data Managment, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

To: docket@bozpat.com,,
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 93726

Feb 09, 2018 03:30:52 AM

Dear PAIR Customer:

EPA - Bozicevic Field & Francis LLP
Bozicevic, Field & Francis
201 REDWOOD SHORES PARKWAY
SUITE 200
REDWOOD CITY, CA 94065
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 93726 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

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Application	Document	Mailroom Date	Attorney Docket No.
15550995	NTC.PUB	02/08/2018	CCPIT-002

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/550,995	08/14/2017	Chaohong Hu	CCPIT-002	1813

93726 7590 04/04/2019

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EXAMINER
CANELLA, KAREN A

ART UNIT	PAPER NUMBER
1643	

NOTIFICATION DATE	DELIVERY MODE
04/04/2019	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docket@bozpat.com

Office Action Summary	Application No. 15/550,995	Applicant(s) Hu, Chaohong	
	Examiner KAREN A CANELLA	Art Unit 1643	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 2-16,23-28 and 35-36 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 2-16,23-28 and 35-36 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☑ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☑ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☑ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

DETAILED ACTION

Notice of Pre-AIA or AIA Status

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Claims 1, 17-22 and 29-34 have been canceled. Claims 2, 5, 7,-12, 15, 16, 23-25, 27 and 28 have been amended. Claims 35 and 36 have been added. Claims 2-16, 23-28, 35 and 36 are pending and examined on the merits.

Claim Rejections - 35 USC § 112

The following is a quotation of 35 U.S.C. 112(d):

> (d) REFERENCE IN DEPENDENT FORMS.—Subject to subsection (e), a claim in dependent form shall contain a reference to a claim previously set forth and then specify a further limitation of the subject matter claimed. A claim in dependent form shall be construed to incorporate by reference all the limitations of the claim to which it refers.

The following is a quotation of pre-AIA 35 U.S.C. 112, fourth paragraph:

> Subject to the following paragraph [i.e., the fifth paragraph of pre-AIA 35 U.S.C. 112], a claim in dependent form shall contain a reference to a claim previously set forth and then specify a further limitation of the subject matter claimed. A claim in dependent form shall be construed to incorporate by reference all the limitations of the claim to which it refers.

Claims 11 and 12 are rejected under 35 U.S.C. 112(d) or pre-AIA 35 U.S.C. 112, 4th paragraph, as being of improper dependent form for failing to further limit the subject matter of the claim upon which it depends, or for failing to include all the limitations of the claim upon which it depends.

Claim 11 requires, in part, that the cytotoxic agent is selected from a group including "toxins". Claim 12 requires, in part, that the cytotoxic agent is selected from a group including "other toxins". The reciting of a "toxin" or "other toxins" fails to further limit the scope of the cytotoxic agent required in Formula I of claim 10 because all toxins are cytotoxins when delivered by antibody conjugate. Applicant may cancel the claims, amend the claims to place the claims in proper dependent form, rewrite the claims in independent form, or present a sufficient showing that the dependent claims comply with the statutory requirements.

The following is a quotation of 35 U.S.C. 112(b):

> (b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:
The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 11, 12 and 14 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

Claim 11 is vague and indefinite in the inclusion of "cytokines" and "nanoparticles" in a group attributed to be cytotoxic agents. Cytokines are known in the art as proteins secreted by inflammatory leukocytes and some non-leukocytic cells that act as intercellular mediators. As such a cytokine is not a cytotoxic agent. A nanoparticle is refers to the size of a particle. "As such a generic nanoparticle is not a cytotoxic agent. Thus the scope of claim 11 is unclear.

Claim 12 is vague and indefinite in the inclusion of interleukins, chemokines and nanoparticles in a group attributed to be cytotoxic agents. Interleukins are soluble proteins which stimulate growth-related activities of leukocytes as well as other cell types. Chemokines are known in the art as pro-inflammatory cytokines that have the ability to attract and activate leukocytes. As such, an interleukin or a chemokine is not a cytotoxic agent. A nanoparticle is refers to the size of a particle. As such a generic nanoparticle is not a cytotoxic agent. Thus the scope of claim 12 is unclear.

Regarding claim 12, the phrase "and other toxins" renders the claim(s) indefinite because the claim(s) include(s) elements not actually disclosed (those encompassed by "and other toxins"), thereby rendering the scope of the claim(s) unascertainable. See MPEP § 2173.05(d).

Claim 14 is vague and indefinite in the recitation of "preferably 6-maleimidocaproyl....". It is unclear how this preference modulates the scope of the claim.

The following is a quotation of the first paragraph of 35 U.S.C. 112(a):

(a) IN GENERAL.—The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor or joint inventor of carrying out the invention.

The following is a quotation of the first paragraph of pre-AIA 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 2-16, 23-28, 35 and 36 are rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The factors considered when determining if the disclosure satisfies the enablement requirement and whether any necessary experimentation is undue include, but are not limited to: 1) nature of the invention, 2) state of the prior art, 3) relative skill of those in the art, 4) level of predictability in the art, 5) existence of working examples, 6) breadth of claims, 7) amount of direction or guidance by the inventor, and 8) quantity of experimentation needed to make or use the invention. In re wands, 858 F.2d 731, 737.8 USPQ2d 1400, 1404 (Fed. Cir. 1988).

(A) Claims 11 and 12 require, in part, cytotoxic agents which are nanoparticles. When given the broadest reasonable interpretation, nanoparticles encompass inert sub-micron particles. The art recognizes that such particles can accumulate in the tumor vasculature through the enhanced permeability and retention effect and can serve as platforms for drug delivery to the tumor vasculature (Prabhakar et al, Cancer Research, 2013, Vol. 73, pp. 2412-2417). However, said particles alone have no cytotoxic property. The specification fails to provide any objective evidence that "nanoparticles" without an attached cytotoxic moiety would have any toxic effect when internalized by the EGFR conjugate of the claims.

(B) Claims 11 and 12 require in part cytotoxic agent which are cytokines, interleukins and chemokines. Cytokines are known in the art as proteins secreted by inflammatory leukocytes and some non-leukocytic cells that act as intercellular mediators. Interleukins are soluble proteins which stimulate growth-related activities of leukocytes as well as other cell types. Chemokines are known in the art as pro-inflammatory cytokines that have the ability to attract and activate leukocytes. Neither the specification nor the prior art teaches toxic effect of cytokines, interleukins or chemokines on cells expressing EGFR. The specification lacks any

objective evidence that cytokines, chemokines or interleukins, when internalized by the conjugate bind to EGFR on a target cell would exert a toxic effect on the target cell.

(C) The instant claims 2-16, 23-28, 35 and 36 require in part, a solvate of the antibody conjugates. The specification fails to provide any teachings for how to make the solvate of the instant antibody conjugates. Matheus et al (U.S. 7,960,516) teach that the term solvates is taken to mean adductions of inert solvent molecules onto a solid form which adductions form due to their mutual attractive force, and that solvates include hydrates such as monohydrates or dihydrates or alcoholates (column 10, lines 37-43). Matheus et al teach the unexpected result that stable pharmaceuticals can be prepared with solid forms of anti-EGFR antibodies such as cetuximab (column 4, lines 56-59) and that the antibodies are biologically active after re-dissolution (column 10, lines 51-59). Matheus et al teach that the difficulties in crystallizing intact glycosylated antibodies is extremely difficult due to the size of the protein, the different glycosylation patterns of the individual molecules and associated micro-heterogeneities as well as the structural flexibility of the immunoglobulin make an ordered incorporation into a crystal lattice more difficult or even impossible (column 2, lines 50-56) and that there is a risk of denaturing antibodies during the crystallization process (column 2, lines 63-66). The instant claims require a solvate of an anti-EGFR cytotoxin conjugate. The various cytotoxins and linker required in the conjugate would vastly increase the size of the molecule, the flexibility and the micro-heterogeneity, especially since the number of linkers attached to a single antibody are variable. Further some of the cytotoxins only moderately soluble in water, such as doxorubicin, and the influence of a group that is less hydrophilic than an antibody on the ability of the antibody to go into a crystal lattice is unknown. The specification has not provided the conditions for the formation of a single solvate of the large genus of EGFR-conjugates encompassed by the claims. It would not be expected that the conditions disclosed for the formation of hydrates of unconjugated cetuximab would be the same conditions for the formation of hydrates or solvates for the instant genus of anti-EGFR-conjugates claimed. Thus one of skill in the art would be subject to undue experimentation in order to make the solvates of the large genus of anti-EGFR conjugates claimed.

(D) Claims 23, 24, 27 and 28, require in part, a method for prophylaxis of disease associated with EGFR comprising administering a prophylactically effective amount of the antibody-drug conjugate. Claims 25, 26, 35 and 36 require in part a method for inhibiting tumor

angiogenesis, delaying tumor progression, inhibiting tumor growth or inhibiting tumor cell proliferation comprising administering a prophylactically effective amount of the antibody-drug conjugate. When given the broadest reasonable interpretation, "a subject" includes a human or a non-experimental animal. In order to provide prophylaxis, it is necessary to administer the conjugate before the disease occurs. Administration of an antibody-conjugate would have a finite lifetime in the blood and would not be expected to invoke any immunological memory. Thus the antibody must be in the circulating blood prior to the time of emergence of the disease cell. The specification fails to teach how to predict when a disease cell expressing EGFR will occur in a human or a non-experimental animal such that the antibody conjugate can be injected prior to disease occurrence. Further, the specification fails to teach how a "prophylactically effective amount" of the antibody conjugate differs from a "therapeutically effective amount. Given the lack of teachings or guidance regarding these issues, one of skill in the art would be subject to undue experimentation in order to carry out the broadly claimed methods

Claims 2-16, 23-28, 35 and 36 are rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor or a joint inventor, or for pre-AIA the inventor(s), at the time the application was filed, had possession of the claimed invention.

(A) Claims 2-6, 8 encompass "mutants thereof" in regard to the EGFR antibody required by the claims. Claims 7 and 9 encompass sequences having greater than 70% or greater than 75%, 80%, 85%, 90%, 95% or 99% identity to SEQ ID NO:3 or 4. The disclsoure of a single antibody heavy chain variable region, light chain variable region and constant regions does not provide an adequate written description of the genus of antibodies encompassed in the claimed antibody conjugates. The Court in in Amgen v. Sanofi of October 5, 2017 determined that a description of an antibody must be in terms of the total structure of the antibody, or structure of the antibody paratope region responsible for binding to the antigen. In the instant claims, the "mutants thereof" or the alteration in sequence that contributes to less than 100% sequence

identity can be alterations in the CDR regions of the antibody. Thus, the genus of antibodies encompassed by the claims would be made up of antibodies having highly variable paratopes, The description of the instant CDR regions of the heavy chain of SEQ ID NO:5-7 and CDR region of the light chain of SEQ ID NO:12-14 fail to describe the claimed genus because the genus is no longer limited by antibody paratope of the disclosed SEQ ID NO:5-7 and SEQ ID NO:12-14. One of skill in the art would not be able to envisage the claimed genus as one can do with a well described genus. One of skill in the art would reasonably conclude that applicant was not in possession of the claimed genus at the time of filing.

(B) The instant claims all require in part, a solvate of the ANTIBODY CONJUGATE. The specification fails to provide any teachings for how to make the solvate of the instant ANTIBODY CONJUGATEs. Matheus et al (U.S. 7,960,516) teach that the term solvates is taken to mean adductions of inert solvent molecules onto a solid form which adductions form owning to their mutual attractive force, and that solvates include hydrates such as monohydrates or dihydrates or alcoholates (column 10, lines 37-43). Matheus et al teach the unexpected result that stable pharmaceuticals can be prepared with solid forms of anti-EGFR antibodies such as cetuximab (column 4, lines 56-59) and that the antibodies are biologically active after re-dissolution (column 10, lines 51-59). Matheus et al teach that the difficulties in crystallizing intact glycosylated antibodies is extremely difficult due to the size of the protein, the different glycosylation patterns of the individual molecules and associated micro-heterogeneities as well as the structural flexibility of the immunoglobulin make an ordered incorporation into a crystal lattice more difficult or even impossible (column 2, lines 50-56) and that there is a risk of denaturing antibodies during the crystallization process (column 2, lines 63-66). The instant claims require a solvate of an anti-EGFR cytotoxin conjugate. The various cytotoxins and linker required in the conjugate would vastly increase the size of the molecule, the flexibility and the micro-heterogeneity, especially since the number of linkers attached to a single antibody are variable. Further some of the cytotoxins only moderately soluble in water, such as doxorubicin (Yoo and Park, Journal of Controlled Release, 2001, Vol. 70, pp. 63-70, see page 67, second column, line 30), and the influence of a group that is less hydrophilic than an antibody on the ability of the antibody to go into a crystal lattice is unknown. The specification has not provided the conditions for the formation of a single solvate of the large genus of EGFR-conjugates encompassed by the claims. It would not be expected that the conditions disclosed for the

formation of hydrates of unconjugated cetuximab would be the same conditions for the formation of hydrates or solvates for the instant genus of anti-EGFR-conjugates claimed. One of skill in the art would reasonably conclude that applicant was not in possession of the claimed invention at the time of filing.

Claim Rejections - 35 USC § 103

In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

This application currently names joint inventors. In considering patentability of the claims the examiner presumes that the subject matter of the various claims was commonly owned as of the effective filing date of the claimed invention(s) absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and effective filing dates of each claim that was not commonly owned as of the effective filing date of the later invention in order for the examiner to consider the applicability of 35 U.S.C. 102(b)(2)(C) for any potential 35 U.S.C. 102(a)(2) prior art against the later invention.

The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent for a claimed invention may not be obtained, notwithstanding that the claimed invention is not identically disclosed as set forth in section 102, if the differences between the claimed invention and the prior art are such that the claimed invention as a whole would have been obvious before the effective filing date of the claimed invention to a person having ordinary skill in the art to which the claimed invention pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 2-14, 16, 23-26 are rejected under 35 U.S.C. 103 as being unpatentable over Tikhomirov (WO2015/000062) in view of Liu (CN103772504 reference of the IDS filed 8/14/2017), Brand et al (Cancer Biology & Therapy, 2011, Vol. 11, pp. 777-792) and Chung et al (New England Journal of Medicine, 2008, Vol. 358, pp. 1109-1117).

Claim 2 is drawn in part to an antibody-drug conjugate comprising an anti-EGFR antibody covalently linked to a cytotoxic agent, wherein the anti-EGFR antibody comprises the a heavy chain and a light chain, wherein the heavy chain variable region comprises SEQ ID NO:5-7 as the heavy chain CDR1-3, and the light chain variable region comprises SEQ ID NO:12-14 as the light chain CDR1-3, Claim 3 embodies the ANTIBODY CONJUGATE of

claim 1 wherein the FR1-4 of the variable regions of the heavy chain comprises SEQ ID NO:8-11, respectively. Claim 4 embodies the antibody conjugate of claim 1 wherein the FR1-4 of the variable regions of the light chain comprises SEQ ID NO:15-18, respectively. Claim 5 requires that the antibody conjugate of claim 2has a constant region selected from a group including the constant region of human IgG. Claim 6 specifies that the IgG constant region of claim 5 is selected from a group including IgG1. Claim 7 requires, in part, that the antibody conjugate of claim 5 has a constant region comprising SEQ ID NO:3. Claim 8 requires in part that the light chain of claim 2 has a constant region selected from a group including human kappa constant region. Claim 9 requires in part that the light chain constant region of claim 7 comprises SEQ ID NO:4. Claim 10 specifies that the antibody conjugate of claim 2 has the structure of Formula I wherein Ab is the antibody, L is the linker, D is the cytotoxic agent and p is 1-9. Claim 11 requires that the antibody conjugate of claim 10 comprises a toxin or a biological active peptide as the cytotoxin; claim 12 requires, in part, that the cytotoxic agent of claim 11 is MMAE. Claim 13 embodies the antibody conjugate of claim 10 wherein the linker is non-cleavable. Claim 14 requires that the linker of claim 13 is selected from a group including 6-maleimidocaproyl (MC).

Claim 16 is drawn to a composition comprising the antibody conjugate of claim 2 optionally comprising at least one pharmaceutically acceptable carrier, diluent or excipient.

Claim 23 is drawn in part to a method for the treatment of a disease associated with EGFR comprising administering to a subject in need thereof, a therapeutically effective amount of the antibody conjugate of claim 2. Claim 24 requires that the disease associated with EGFR is a tumor with overexpression of EGFR.

Claim 25 is drawn to a method for inhibiting tumor angiogenesis, delaying tumor progression, inhibiting tumor growth or inhibiting tumor cell proliferation comprising administering to a subject in need thereof a therapeutically effective amount of the antibody conjugate of claim 2. Claim 26 embodies the method of claim 25 wherein the tumor is selected from colon cancer, rectal cancer, head and neck cancer, lung cancer, ovarian cancer, cervical cancer, bladder cancer, esophageal cancer, breast cancer, renal cancer, prostate cancer, gastric cancer, pancreatic cancer and brain glioma.

Tikhomirov et al teach method for treating a subject presenting with a tumor that responds to treatment with a full antagonist EGFR antibody, wherein treatment therewith elicits

an EGFR antibody-mediated adverse response by keratinocytes, the improvement comprising treating said subject with the full antagonist EGFR antibody in a form conjugated with an anti-microtubule toxin, whereby the tumor response to treatment with conjugated antibody is enhanced essentially without enhancing the adverse keratinocyte response to treatment, relative to treatment with naked antibody alone (claim 19 of '062) thus teaching the embodiments of instant clams23 and 25. Tikhomirov et al teach that a preferred embodiment of the full antagonist EGFR antibody is cetuximab, but that the invention includes other antibodies which are not cetuximab; the preferred anti-microtubulin toxin is preferably an auristatin (page 15, lines 14-16) and the linker is preferably a non-cleavable linker and preferably non—cleavable malimidocaproyl linkers for linking auristatins as the anti-microtubule toxin (page 14, lines 5-7) and that conjugation of cetuximab to MMAE anti-microtubule payload by a cleavable linker potentiates its toxicity against both normal cells and MDA-MB-468 cancer cells, whereas conjugation via non-cleavable linker only potentiates anti-cancer activity, thus demonstrating a favorable therapeutic selectivity (page 9, line 24 to page 10, line 3) thus teaching the embodiments of claims 10-12 wherein the cytotoxin is MMAE, the linker linking the MMAE to the antibody is non-cleavable and maleimidocaproyl (MC). Tikhomirov et al teach pharmaceutical compositions comprising the EGFR immunoconjugates in a pharmaceutically acceptable carrier, thus teaching the limitation of claim 16.

Tikhomirov et al also teach a less preferred embodiment of a cleavable linker (page 6, lines 3-5) such as a linker incorporating valine-citrulline such as cetux-2C9-MMAE in Figure 13, which meets the specific embodiment of cleavable linker in instant claim 13.

Tikhomirov et al teach the that the subject presenting with EGFR+ cancer cells are particularly EGFR+ cells having on their surface an abnormally high density of EGFR molecules (page 18, lines 1-2) which meets the limitation of instant claim 24 requiring EGFR overexpression. Tikhomirov et al teach the that cancers treatable by the invention include EGFR+ colo-rectal cancer, head and neck cancer, lung cancer, ovarian cancer, cervical cancer, bladder cancer, esophageal cancer, breast cancer, renal cancer, prostate cancer, gastric cancer, pancreatic cancer and glioblastoma (page 18, lines 10-25) which meets the limitation of claim 26.

As noted above, Tikhomirov et al teach the preferred embodiment of cetuximab as the anti-EGFR antibody of the invention. Tikhomirov et al do not teach an anti-EGFR having the required CDRs, variable regions, and constant regions set forth in claims 2-8.

Brand et al teach that cetuximab, also known as C225, is the chimeric version of the murine antibody M225 (page 781, Figure 2).

Chung et al (NEJM, 2008, Vol. 358, pp. 1109-1117) teach that among 76 cetuximab treated subjects, 25 had hypersensitivity reactions to the drug, and that most of the subjects having hypersensitivity reactions had pre-existing IgE antibodies to galactose-α-1,3-galactose which is present on the Fab portion of the cetuximab heavy chain (page 1109, under the heading of "Results"). Chung et al teach that such IgE antibodies are prevalent in the population in areas where anaphylactic reactions to cetuximab occur (page 1110, first column, last sentence before "Methods"). Chung et al teach that the Fab portion of the cetuximab heavy chain is glycosylated at residue N88 with a range of sugars including galactose-α-1,3-galactose (page 1114, second column, lines 11-15).

Liu et al teach four humanized versions of the murine M225 antibody (Translation, page10, line 20 to the end of page 11) comprising a human IgG1 Fc fragment and a kappa light chain, wherein the antibodies are termed BA03, BB03, BC03 and BD03 (translation, page 12, lines 1-6). Liu et al teach the sequence of the heavy chain variable region, wherein the N88 residue is in the FR3 domain of SEQ ID NO:17 (Translation, page 15, last paragraph, CN103772504, page 11, paragraph [0083], the "N" in QSNDT) Liu et al teach the heavy chain variable regions of BA03, BB03, BC03 and BD03, wherein SEQ ID NO:5 of the BA03 heavy chain(translation page 16, SEQ ID NO:5) comprises SEQ ID NO: 5-7 of the instant heavy chain CDRs in addition to the framework domains FR1, FR2, FR3 and FR4 comprising SEQ ID NO:8-11; SEQ ID NO: 13 of the light chain of BA03 (page 19 of the translation, SEQ ID NO:13) comprises the instant CDRs of SEQ ID NO:12-14 and the light chain framework sequences of the instant SEQ ID NO: 15-18.

The kappa light chain of the constant region (Translation, page 21, SEQ ID NO:43) is identical to the instant SEQ ID NO:4:

unnamed protein product
Sequence ID: Query_215689Length: 107Number of Matches: 1

```
Query  1    RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD  60
            RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
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Sbjct  1    RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD  60

Query  61   SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC  107
            SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
Sbjct  61   SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC  107
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The IgG1 Fc fragment (Translation, page 21, SEQ ID NO: 42) is identical to the instant SEQ ID NO:3

unnamed protein product
Sequence ID: Query_227965Length: 330Number of Matches: 1

```
Query  1    ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS  60
            ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
Sbjct  1    ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS  60

Query  61   GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGG  120
            GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGG
Sbjct  61   GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGG  120

Query  121  PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN  180
            PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
Sbjct  121  PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN  180

Query  181  STYRVVSVITVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE  240
            STYRVVSV+TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE
Sbjct  181  STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE  240

Query  241  MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW  300
            MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
Sbjct  241  MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW  300

Query  301  QQGNVFSCSVMHEALHNHYTQKSLSLSPGK  330
            QQGNVFSCSVMHEALHNHYTQKSLSLSPGK
Sbjct  301  QQGNVFSCSVMHEALHNHYTQKSLSLSPGK  330
```

Liu teaches that the BA03 an BD03 antibodies have higher binding affinity, stronger ligand blocking ability and stronger inhibitory activity against surface EGFR than Erbitux/cetuximab, and that BB03 and BC03 have binding affinity and ligand blocking abilities that are similar to that of Erbitux.

It would have been prima facie obvious as of the effective filing date to use the BA03 antibody in place of cetuximab in the conjugates and methods of using the conjugates of Tikhomirov et al. One of skill in the art would have been motivated to do so because the BA03 antibody is a full length antibody that is an antagonist of EGFR as demonstrated by Liu and has higher binding affinity and ligand blocking abilities than cetuximab. One of skill in the art

would also be motivated to use the humanized BA03 antibody in place of cetuximab because the BA03 has humanized framework regions, and because the N88 amino acid in the chimeric 225 antibody is no longer present. Thus, one of skill in the art would know that the humanized 225 antibody of Liu BA03 would not carry galactose-α-1,3-galactose on N88 within FR3, and the antibody would not cause hypersensitivity reactions in subjects having pre-existing IgE antibodies to galactose-α-1,3-galactose.

Claims 2-16, 23-26 are rejected under 35 U.S.C. 103 as being unpatentable over Tikhomirov et al , Liu and Chung et al as applied to claims 2-14, 16, 23-26 above, and further in view of Chari et al (Angewandte Chemie, 2014, Vol. 53, pp. 3796-3827, see page 3811).

Claim 15 requires the antibody linked by means of a sulfonyl group to 6-malimidocaprolyl-valine-citrulline-p-aminobenzyloxycarbonyl to MMAE, wherein p is 1-8.

Tikhomirov et al teach the linkage of MMAE to auristatins by means of maleimidocaprolyl linkers (page 14, lines 5-7) and cleavable linkers comprising valine- citrulline as less preferred embodiments (page 6, lines 3-5). Tikhomirov et al do not specifically teach the structure of claim 15.

Chari et al teach a Mal-caproyl-val-cit-PAB-MMAE linkable derivate of MMAE (middle of page 3811), and the linkage to a mAb, wherein cleavage at the cittrulene induces the self-immolation of the p-amino benzoate to release free MMAE (page 3812, top of page). Chari et al teach that the interchain disulfides can be fully or partially reduced by a mild reducing agent to give a distribution of antibody species with two to eight free thiol groups which react with a maleimide comprising auristatin derivative to give conjugates with an average of four auristatins linked per antibody (page 3811, second column, line 3 to page 3812, first column, line 7). Chari et al teach that with the auristatin MMAF, the val-cit linked MMAF can be up to 10 times more potent on some cell types versus the MMAF with a non-cleavable linker (page 3812, first column, lines 14-17).

It would have been prima facie obvious at the time of the effective filing date to make the antibody maleimidocarpoly-val-cit-PAB-MMAE of Chari et al. One of skill in the art would have been motivated to do so because Tikhomirov et al teach the linkage of MMAE to auristatins by means of maleimidocaprolyl linkers (page 14, lines 5-7) and cleavable linkers comprising

valine-citrulline and because Chari et al teach in some cases, a val-cit linked carrying an auristatin is more potent than a auristatin antibody conjugate with a non-cleavable linker.

Claims 2-14, 16, 23-27 and 35 are rejected under 35 U.S.C. 103 as being unpatentable over Tikhomirov et al, Liu and Chung et al as applied to claims 2-14, 16, 23-26 above, and further in view of Lievre et al (Cancer Research, 2006, Vol. 66, pp. 3992-3995).

Claims 27 and 35 embody the methods of claims 24 and 26, respectively, wherein the tumor is a tumor with a KRAS mutation.

The combination of Tikhomirov et al, Liu and Chung et al render obvious claims 2-14, 16, 23-26 for the reasons set forth above. None of Tikhomirov et al, Liu or Chung et al teach the treatment of subject having an EGFR positive tumor with a KRAS gene mutation.

Lievre et al teach that patients with KRAS tumors are resistant to cetuximb (abstract, penultimate sentence). Lievre et al teach that the whatever the expression level of EGFR is, the presence of the KRAS mutation is associated with a downstream activation of the Ras/MAPK pathway leading to cell proliferation that cannot be significantly inhibited by cetuximab, because cetuximab acts upstream of the K-ras protein (page 3995, second column, lines 10-14).

It would have been prima facie obvious at the time of the effective filing date to treat patients with EGFR expressing tumors and KRAS mutations with the antibody conjugate rendered obvious by the combination of Tikhomirov et al, Liu and Chung et al. One of skill in the art would have been motivated to do so because one of skill in the art would know that the conjugate would have a mechanism of action that differed from the administration of the unconjugated cetuximab. One of skill in the art would understand that the conjugate would be internalized and the cytotoxic moiety would escape from the lysosomal compartment to deliver the cytotoxin intracellularly and that this cytotoxin would be toxic to cells harboring the KRAS mutation even in the event that the Ras/MAPK pathway would be potentially activated by binding of the conjugated BA03 antibody.

Claims 2-14, 16, 23-26, 28 and 36 are rejected under 35 U.S.C. 103 as being unpatentable over Tikhomirov et al , Liu and Chung et al as applied to claims 2-14, 16, 23-26 above, and further in view of DiNicolantonio et al (Journal of Clinical Oncology, 2008, Vol. 26, pp. 5705-5712).

Claims 28 and 36 embody the methods of claims 24 and 26, respectively, wherein the tumor is a tumor with a BRAF mutation.

The combination of Tikhomirov et al, Liu and Chung et al render obvious claims 2-14, 16, 23-26 for the reasons set forth above. None of Tikhomirov et al, Liu or Chung et al teach the treatment of subject having an EGFR positive tumor with a BRAF mutation.

DiNicolantonio et al teach that patients with BRAF mutated EGFR expressing tumors did not respond to cetuximab (page 5705 under "Results" and page 5706, second column, lines 6-8). DiNicolantonio et al teach that the therapeutic effect of cetuximab could be restored if administered with a BRAF inhibitor such as sorafenib or other compounds targeting BRAF or its downstream effectors.

It would have been prima facie obvious at the time of the effective filing date to treat patients with EGFR expressing tumors and BRAF mutations with the antibody conjugate rendered obvious by the combination of Tikhomirov et al, Liu and Chung et al. One of skill in the art would have been motivated to do so because one of skill in the art would know that the conjugate would have a mechanism of action that differed from the administering of the unconjugated cetuximab. One of skill in the art would understand that the conjugate would be internalized and the cytotoxic moiety would escape from the lysosomal compartment to deliver the cytotoxin intracellularly and that this cytotoxin would be toxic to cells harboring the BRAF mutation even in the event that the downstream effectors of BRAF would be activated.

All claims are rejected.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to KAREN A CANELLA whose telephone number is (571)272-0828. The examiner can normally be reached on M-F 10-6:30.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is

encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Misook Yu can be reached on 571-272-0839. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

KAREN A. CANELLA
Examiner
Art Unit 1643

/Karen A. Canella/
Primary Examiner, Art Unit 1643

Notice of References Cited	Application/Control No. 15/550,995	Applicant(s)/Patent Under Reexamination Hu, Chaohong	
	Examiner KAREN A CANELLA	Art Unit 1643	Page 1 of 2

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-7960516-B2	06-2011	Matheus; Susanne	A61K39/39591	530/387.3
	B					
	C					
	D					
	E					
	F					
	G					
	H					
	I					
	J					
	K					
	L					
	M					

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N	WO-2015000062-A1	01-2015	WO	TIKHOMIROV ILIA ALEXANDRE	A61K47/50
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Prabhakar et al, Cancer Research, 2013, Vol. 73, pp. 2412-2417 (Year: 2013)
	V	Yoo and Park, Journal of Controlled Release, 2001, Vol. 70, pp. 63-70 (Year: 2001)
	W	Brand et al (Cancer Biology & Therapy, 2011, Vol. 11, pp. 777-792) (Year: 2011)
	X	Chung et al (New England Journal of Medicine, 2008, Vol. 358, pp. 1109-1117) (Year: 2008)

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Notice of References Cited	Application/Control No. 15/550,995	Applicant(s)/Patent Under Reexamination Hu, Chaohong	
	Examiner KAREN A CANELLA	Art Unit 1643	Page 2 of 2

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
	A					
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FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Chari et al (Angewandte Chemie, 2014, Vol. 53, pp. 3796-3827) (Year: 2014)
	V	Lievre et al (Cancer Research, 2006, Vol. 66, pp. 3992-3995) (Year: 2006)
	W	DiNicolantonio et al (Journal of Clinical Oncology, 2008, Vol. 26, pp. 5705-5712) (Year: 2008)
	X	EPO Translation of CN103772504, downloaded from the Web on 3-16-2019 (Year: 2019)

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/550,995	Hu, Chaohong
	Examiner	Art Unit
	KAREN A CANELLA	1643

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
STN	03/16/2019	kac
West	03/31/2019	kac
inventor	03/31/2019	kac

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

/Karen A. Canella/ Primary Examiner, Art Unit 1643	

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DESCRIPTION CN 103772504

Abstract The present invention relates to a humanized anti-epidermal growth factor receptor antibody. The invention also relates to nucleic acid molecules encoding the antibodies, vectors, host cells and compositions comprising the nucleic acid molecules, and detection reagents and kits. The invention also relates to the use of the antibody for the preparation of a medicament for the prevention/treatment of epidermal growth factor or epidermal growth factor receptor-associated diseases and for the preparation of a detection reagent or kit. The antibody molecule of the invention has lower immunogenicity and higher target binding activity, thereby having stronger biological activity and anti-tumor activity in vitro and in vivo, and can be expected to have lower side effects and better curative effect in clinical application.

Humanized anti-epidermal growth factor receptor antibody and use thereof

Technical field

The present invention relates to a humanized antibody, in particular to a humanized anti-epidermal growth factor receptor antibody, and to the use of the antibody for the preparation of a medicament for preventing or treating a disease associated with high activity or high expression of epidermal growth factor. Compared with human and mouse chimeric antibodies, the humanized anti-epidermal growth factor antibody of the present invention further reduces the immunogenicity of the antibody, and enhances its antigen recognition activity and anti-tumor function in vitro and in vivo, and has better clinical performance. Application prospects.

Background technique

Epidermal Growth Factor Receptor (EGFR, also known as HER1, c-ErbB1) is a cell surface receptor of the epidermal growth factor family, which is composed of 1186 amino acid residues and has a molecular weight of 170 kD. Glycoprotein (Jorissen RN, Walker F, Pouliot N, et al. Epidermal growth factor receptor: mechanisms of activation and signaling. Exp Cell Res, 2003; 284: 31-53). EGFR belongs to the type I tyrosine kinase receptor ErbB subfamily (ErbB 1-4) and has tyrosine kinase activity. EGFR is stably expressed in many epithelial tissues, including skin and hair follicles. Abnormal expression of the epidermal growth factor receptor or activation by receptor mutations can lead to cancer. There are many solid tumors found to express high expression of epidermal growth factor receptors, such as colorectal cancer, head and neck cancer, lung cancer, ovarian cancer, cervical cancer, bladder cancer and esophageal cancer (Olayioye MA, Neve RM, Lane HA, et al. The ErbB Signaling network: receptor heterodimerization in development and cancer. The EMBO J, 2000; 19: 3159-3167). Growth factors such as transforming growth factor alpha and epidermal growth factor are endogenous ligands for the epidermal growth factor receptor. These ligands bind to the epidermal growth factor receptor, activate the activity of the receptor intracellular tyrosine protein kinase, initiate multiple downstream signal transduction pathways, thereby regulating the growth and differentiation of normal cells, increasing the invasiveness and promotion of tumor cells. Angiogenesis, inhibition of tumor cell apoptosis (Ciardiello F, Tortola G A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. Clin Cancer Res, 2001; 7: 2958-2970). The high expression of epidermal growth factor receptor in tumors and its important role in tumor cell growth and differentiation make epidermal growth factor receptor a promising tumor therapeutic target.

The therapeutic antibody against the epidermal growth factor receptor was originally derived from the murine antibody M 225 obtained by immunizing mice with epidermal growth factor receptor high expression epidermal carcinoma A 431. M 225 specifically binds to the epidermal growth factor receptor and blocks its binding to the ligand, thereby inhibiting the activity of the epidermal growth factor receptor tyrosine protein kinase (Goldstein et al, 1995, Clin. Cancer. Res. 1: 1311-1318). As a foreign protein, the murine antibody repeatedly stimulates the human body and can cause a hypersensitivity reaction, which is called a human anti-mouse antibody reaction. The murine antibody may be neutralized by anti-antibodies in the body, which is rapidly cleared and even causes a severe allergic reaction to death. In addition, murine monoclonal antibodies lack the immunological effector functions of human antibodies, such as antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), and thus are not effective. Thus, the human murine chimeric antibody C 225 antibody (Erbitux, Erbitux or Cetuximab, ImClone (now EliLilly)) uses the variable regions of human IgG 1 kappa and murine anti-M 225 to reduce the immunogenicity of murine mAbs, retaining Specific affinity with epidermal growth factor receptor can block the binding of ligands such as EGF and TGFa to epidermal growth factor receptor, inhibit its phosphorylation and downstream signaling, thereby inhibiting tumor cell growth and inducing apoptosis. Reduce the production of matrix metalloproteinases and vascular epithelial growth factors. 2004 The US FDA approved Erbitux for colorectal cancer, and 2006 approved for the treatment of head and neck cancer. There are more clinical trials for other cancer indications. Compared with other chemotherapeutic drugs, antibody drugs have strong specificity and small side effects, and have achieved good clinical results. However, human and mouse chimeric Erbitux imab are still immunogenic, clinically reported to have an immunogenicity of 5%, which is prone to human anti-antibody reaction leading to immune complex-mediated antibody clearance, and skin and mucosal side effects. (An Z, et al., 2009, Therapeutic

Monoclonal Antibody, p313).

Panitumumab (Amgen) is a fully humanized monoclonal antibody prepared using transgenic mouse technology. It has no murine protein sequence and targets the epidermal growth factor receptor (EGFR). It was approved by the FDA in September 2006. Approved for marketing, in combination with fluoropyrimidine, oxaliplatin and irinotecan or after chemotherapy for the treatment of EGFR-positive metastatic colorectal cancer. 2006 The FDA approved its monotherapy for chemotherapy-resistant metastatic colorectal cancer (mCRC). However, panitumumab is an IgG2 subtype antibody. Compared with IgG1, the CDC activity of IgG2 and the biological activity of ADCC are significantly reduced. In addition, the stability of IgG2 subtype antibody is poor, which may be a clinical effect on fully human antibody. The main reason why nimitame has no significant advantage compared to the chimeric antibody Erbitux.

Therefore, there is a need in the art for humanized anti-epidermal growth factor receptor antibodies with lower immunogenicity and higher biological activity to further improve efficacy and reduce side effects.

Summary of the invention

The present inventors have surprisingly obtained a humanized anti-epidermal growth factor receptor antibody having lower immunogenicity and higher biological activity by using antibody humanization technology on the basis of a mouse antibody. The present invention has been completed based on the above findings. Specifically, it includes the following aspects:

A first aspect of the invention relates to an anti-epidermal growth factor receptor antibody or antigen-binding portion thereof, wherein the sequence of the heavy chain CDR1 is selected from the sequences set forth in SEQ ID NO:1 and SEQ ID NO:39 or a derivative thereof, and the sequence of CDR3 is selected a sequence set forth in SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:40 or a sequence derived therefrom; and/or

The sequence of its light chain CDR3 is selected from the sequences set forth in SEQ ID NO:9, SEQ ID NO:10 and SEQ ID NO:41 or derivatives thereof.

The antibody or antigen-binding portion thereof according to the first aspect of the invention, the sequence of the heavy chain CDR2 and the light chain CDR1, CDR2 may be a corresponding portion of a murine or human or human murine chimeric anti-epidermal growth factor receptor antibody.

The antibody or antigen-binding portion thereof according to any one of the first aspects of the invention, the sequence of the heavy and light chain FR thereof may be a corresponding portion of a murine or human or human murine chimeric anti-epidermal growth factor receptor antibody.

In an embodiment of the invention, the sequence of the heavy chain variable region CDR of the antibody or antigen binding portion thereof is selected from the sequences shown in the following (1) to (4) or a derivative thereof:

(1)

The sequence of CDR1 is the sequence shown in SEQ ID NO:1, the sequence of CDR2 is the sequence shown in SEQ ID NO:4, and the sequence of CDR3 is the sequence shown in SEQ ID NO:2.

(2)

The sequence of CDR1 is the sequence shown in SEQ ID NO:1, the sequence of CDR2 is the sequence shown in SEQ ID NO:4, and the sequence of CDR3 is the sequence shown in SEQ ID NO:40;

(3)

The sequence of CDR1 is the sequence shown in SEQ ID NO:39, the sequence of CDR2 is the sequence shown in SEQ ID NO:4, and the sequence of CDR3 is the sequence shown in SEQ ID NO:3;

(4)

The sequence of CDR1 is the sequence of SEQ ID NO:39, the sequence of CDR2 is the sequence of SEQ ID NO:4, the sequence of CDR3 is the sequence of SEQ ID NO:40; and/or

The sequence of the light chain variable region CDR is selected from the sequences shown in the following (5) to (8) or a derivative thereof:

(5)

The sequence of CDR1 is the sequence shown in SEQ ID NO:11, the sequence of CDR2 is the sequence shown in SEQ ID NO:12, and the sequence of CDR3 is the sequence shown in SEQ ID NO:9.

(6)

The sequence of CDR1 is the sequence shown in SEQ ID NO:11, the sequence of CDR2 is the sequence shown in SEQ ID NO:12, and the sequence of CDR3 is the sequence shown in SEQ ID NO:41;

(7)

The sequence of CDR1 is the sequence shown in SEQ ID NO:11, the sequence of CDR2 is the sequence shown in SEQ ID NO:12, and the sequence of CDR3 is the sequence shown in SEQ ID NO:41;

(8)

The sequence of CDR1 is the sequence shown in SEQ ID NO:11, the sequence of CDR2 is the sequence shown in SEQ ID NO:12, and the sequence of CDR3 is the sequence shown in SEQ ID NO:10.

The antibody or antigen-binding portion thereof according to any one of the first aspects of the invention, wherein the sequence of the heavy chain FR1 is selected from the sequence set forth in SEQ ID NO:19 to SEQ ID NO:22 or a derivative thereof, and the sequence of FR2 is selected from the group consisting of SEQ ID NO:23 to SEQ ID NO:25 or a sequence derived therefrom, the sequence of FR3 is selected from the sequence shown in SEQ ID NO:26 to SEQ ID NO:29 or a derivative thereof, and the sequence of FR4 is SEQ ID NO: a sequence of 30 or a sequence derived therefrom; and/or

The sequence of the light chain FR1 is selected from the sequence shown in SEQ ID NO:31 to SEQ ID NO:32 or a sequence derived therefrom, and the sequence of FR2 is selected from the sequence shown in SEQ ID NO:33 to SEQ ID NO:34 or a derivative thereof. The sequence of FR3 is selected from the sequence set forth in SEQ ID NO:35 to SEQ ID NO:36 or a sequence derived therefrom, and the sequence of FR4 is selected from the sequence set forth in SEQ ID NO:37 to SEQ ID NO:38 or a derivative thereof.

In an embodiment of the invention, the sequence of the heavy chain variable region FR of the antibody or antigen-binding portion thereof is selected from the sequences shown in the following (9) to (12) or a derivative thereof:

(9)

The sequence of FR1 is the sequence shown in SEQ ID NO:19, the sequence of FR2 is the sequence shown in SEQ ID NO:23, the sequence of FR3 is the sequence shown in SEQ ID NO:26, and the sequence of FR4 is SEQ ID NO:30. The sequence shown;

(10)

The sequence of FR1 is the sequence shown in SEQ ID NO:20, the sequence of FR2 is the sequence shown in SEQ ID NO:24, the sequence of FR3 is the sequence shown in SEQ ID NO:27, and the sequence of FR4 is SEQ ID NO:30. The sequence shown;

(11)

The sequence of FR1 is the sequence shown in SEQ ID NO:21, the sequence of FR2 is the sequence shown in SEQ ID NO:23, the sequence of FR3 is the sequence shown in SEQ ID NO:28, and the sequence of FR4 is SEQ ID NO:30. The sequence shown;

(12)

The sequence of FR1 is the sequence shown in SEQ ID NO:22, the sequence of FR2 is the sequence shown in SEQ ID NO:25, the sequence of FR3 is the sequence shown in SEQ ID NO:29, and the sequence of FR4 is SEQ ID NO:30. The sequence shown; and/or

The sequence of the light chain variable region FR is selected from the sequences shown in the following (13) to (16) or a derivative thereof:

(13)

The sequence of FR1 is the sequence shown in SEQ ID NO:31, the sequence of FR2 is the sequence shown in SEQ ID NO:33, the sequence of FR3 is the sequence shown in SEQ ID NO:35, and the sequence of FR4 is SEQ ID NO:37. The sequence shown;

(14)

The sequence of FR1 is the sequence shown in SEQ ID NO:31, the sequence of FR2 is the sequence shown in SEQ ID NO:33, the sequence of FR3 is the sequence shown in SEQ ID NO:35, and the sequence of FR4 is SEQ ID NO:38. The sequence shown;

(15)

The sequence of FR1 is the sequence shown in SEQ ID NO:32, the sequence of FR2 is the sequence shown in SEQ ID NO:34, the sequence of FR3 is the sequence shown in SEQ ID NO:36, and the sequence of FR4 is SEQ ID NO:38. The sequence shown;

(16)

The sequence of FR1 is the sequence shown in SEQ ID NO:31, the sequence of FR2 is the sequence shown in

SEQ ID NO:33, the sequence of FR3 is the sequence shown in SEQ ID NO:35, and the sequence of FR4 is SEQ ID NO:37. The sequence shown.

In a particular embodiment of the invention, the heavy chain variable region sequence of the antibody or antigen binding portion thereof is the sequence set forth in SEQ ID NO:5, and the light chain variable region sequence is represented by SEQ ID NO: sequence.

In another specific embodiment of the invention, the heavy chain variable region sequence of the antibody or antigen binding portion thereof is the sequence set forth in SEQ ID NO:6, and the light chain variable region sequence is set forth in SEQ ID NO: The sequence shown.

In another specific embodiment of the invention, the heavy chain variable region sequence of the antibody or antigen binding portion thereof is the sequence set forth in SEQ ID NO:7, and the light chain variable region sequence is set forth in SEQ ID NO: The sequence shown.

In another specific embodiment of the invention, the heavy chain variable region sequence of the antibody or antigen binding portion thereof is the sequence set forth in SEQ ID NO:8, and the light chain variable region sequence is set forth in SEQ ID NO: The sequence shown.

The antibody or antigen binding portion thereof according to any of the first aspects of the invention, which further comprises a human IgG1 heavy chain constant region and a human kappa light chain constant region.

In the present invention, the IgG1 heavy chain constant region includes various allotypes such as G1m(f), G1m(z), G1m(z,a) or G1m(z,a,x).

In an embodiment of the invention, the IgG1 heavy chain constant region is of the G1m(z) form.

In the present invention, the kappa light chain constant region comprises various allotypes, such as Km1, Km1,2 or Km3, and in an embodiment of the invention, the kappa light chain constant region is of the Km3 type.

The antibody or antigen-binding portion thereof according to any of the first aspects of the invention, wherein the antigen binding portion comprises a single chain antibody (scFv), a chimeric antibody, a diabody, a scFv-Fc

bivalent molecule, a dAb and a complementarity determining region (CDR) fragments, Fab fragments, Fd fragments, Fab' fragments, Fv and F(ab')2 fragments.

A second aspect of the invention relates to an isolated nucleic acid molecule encoding an antibody or antigen binding portion thereof according to any of the first aspects of the invention.

A third aspect of the invention relates to a vector comprising the nucleic acid molecule of any of the second aspect of the invention.

In the present invention, the vector may be a cloning vector or an expression vector.

The expression vector is, for example, a prokaryotic expression vector, a eukaryotic expression vector, a phage vector or a viral vector, all of which are commonly used in the art.

Wherein the prokaryotic expression vector is, for example, PET28a, pGEM Ex1, pGEM 7ZF(+) or pBAD24, and the eukaryotic expression vector is, for example, pBudCE4.1, pcDNA3.1, pCMV-Tag2B or pGL3 basic, etc., in the practice of the present invention In the embodiment, the eukaryotic expression vector is pcDNA3.1, and the viral vector is, for example, a retrovirus, a lentivirus, an adenovirus, and an adeno-associated virus.

In an embodiment of the invention, the viral vector is an adenoviral vector.

A fourth aspect of the invention relates to a host cell comprising the nucleic acid molecule of the second aspect of the invention or the vector of the third aspect.

In the present invention, the host cell may be a prokaryotic cell or a eukaryotic cell.

The eukaryotic cell is, for example, a mammalian cell.

The host cell can be obtained by introducing/transfecting the above nucleic acid molecule or vector into a prokaryotic cell or a eukaryotic cell.

In the present invention, the prokaryotic cell may be, for example, Escherichia coli, and the eukaryotic cell may be, for example, a yeast cell, an insect cell (such as Sf21), or a mammalian cell, and the mammal may be, for example, an NS0 cell or a CHO cell.

In one embodiment of the invention, the host cell is a eukaryotic cell.

In one embodiment of the invention, the eukaryotic cell is a CHO-S cell.

In the present invention, host cells transfected with a particular nucleic acid or vector can be obtained using any of a variety of transfection methods known in the art.

For example, nucleic acids can be introduced into cells by electroporation or microinjection.

Alternatively, lipofection reagents such as FuGENE6, X-tremeGENE and LipofectAmine can be used.

Alternatively, the nucleic acid can be introduced into the cell by a suitable viral vector based on retrovirus, lentivirus, adenovirus, and adeno-associated virus.

A fifth aspect of the invention relates to a composition comprising an antibody or antigen-binding portion thereof according to any one of the first aspects of the invention, and optionally one or more pharmaceutically acceptable excipients; preferably, the excipient is selected from the group consisting of Proteolytic enzyme inhibitors, pharmaceutically acceptable carriers, diluents and adjuvants.

A sixth aspect of the invention relates to the antibody or antigen-binding portion thereof according to any one of the first aspects of the invention, or the nucleic acid molecule of the second aspect, or the vector of the third aspect, or the host cell of the fourth aspect, in the preparation of a prophylactic/therapeutic epidermis Use in growth factors or drugs for epidermal growth factor receptor-associated diseases.

In the present invention, the epidermal growth factor or epidermal growth factor receptor-associated disease refers to a disease caused by high expression of epidermal growth factor or high expression of epidermal growth factor receptor.

In an embodiment of the present invention, the epidermal growth factor or epidermal growth factor receptor-associated disease refers to a malignant tumor, which mainly refers to a malignant tumor which occurs in epithelial tissues, including but not limited to colon cancer, liver cancer, and head. Neck cancer, lung cancer, ovarian cancer, cervical cancer, vaginal cancer, bladder cancer, esophageal cancer, oral cancer, skin cancer, breast cancer, prostate cancer and pancreatic cancer.

A seventh aspect of the invention relates to a detection reagent or kit comprising the antibody or antigen-binding portion thereof according to any one of the first aspects of the invention;

Preferably, the antibody or antigen binding portion thereof may further comprise a detectable label; or

Preferably, the detection reagent or kit may further comprise a second antibody that specifically recognizes the antibody or antigen-binding portion thereof;

Preferably, the second antibody may further comprise a detectable label.

An eighth aspect of the invention relates to the use of an antibody or antigen-binding portion thereof according to any of the first aspects of the invention for the preparation of a detection reagent or kit, wherein the detection reagent or kit is for detecting epidermal growth factor or epidermal growth factor Receptors, or used to diagnose epidermal growth factor or epidermal growth factor receptor related diseases.

The invention is further described below.

The object of the present invention is to modify the CDR regions and/or framework regions of antibodies by using de-immunogenic antibody humanization techniques against existing anti-EGFR antibodies to obtain lower immunogenicity and higher A biologically active anti-epidermal growth factor receptor antibody.

In one embodiment of the invention, the sequence of the heavy chain variable region of murine anti-M 225 is first analyzed, and the human antibody gene sequence library is compared according to its heavy chain variable region FR1, FR2, FR3 and FR4 sequences to find out The murine anti-M 225 heavy chain variable region FR1, FR2, FR3 and FR4 sequences are similar to the human germline antibody (lower immunogenicity than the

mature antibody) variable region corresponding sequence.

Then, the indium method was used to analyze the affinity of the similar sequence to the HLA-DR molecule, and the least affinity framework sequence was selected to finally establish the humanized sequence of FR1, FR2, FR3 and FR4 of the heavy chain variable region..

Based on this framework structure, the spatial region of the variable region of murine M225 antibody was analyzed by computer molecular model, and the corresponding amino acid residues of the murine M225 antibody required to support the CDR conformation were analyzed.

De-immunization of CDR1 and CDR3 with high affinity for HLA-DR binding, molecular docking, analysis of epidermal growth factor receptor antigen mouse M225 antibody target and its surrounding amino acid residues and humanized weight The stereoscopic spatial binding of the variable region of the chain, by calculating electrostatic forces, solution mediation (salvation), van der Waals gravitation and entropy values, selecting amino acids that can be optimized in the CDR1 and CDR3 sequences to reduce the affinity of HLA-DR molecules. And improve the binding of the CDR3 region of the antibody to the epitope of the epidermal growth factor receptor.

In another embodiment of the invention, the sequence of the light chain variable region of a murine anti-M225 antibody is analyzed, based on its light chain variable region FR1, FR2, FR3 and FR4 sequences against a human antibody gene sequence library (NCBI IgBLAST) For comparison, we found the corresponding sequence of the human germline antibody variable region similar to the murine anti-M225 light chain variable region FR1, FR2, FR3 and FR4 sequences, and then analyzed the similar sequence and HLA-DR molecule by in silicon. Based on the affinity of the binding, the framework sequence with the lowest affinity was selected, thereby finally establishing the humanized sequences of FR1, FR2, FR3 and FR4 of the light chain variable region.

Based on this framework structure, the spatial region of the variable region of murine 225 antibody was analyzed by computer molecular model, and the corresponding amino acid residues of the murine M225 antibody required to support the CDR conformation were analyzed. De-immunization of CDR1 and CDR3 with high affinity for HLA-DR binding, molecular docking, analysis of epidermal growth factor receptor antigen mouse M225 antibody target and its surrounding amino acid residues and humanized weight The stereoscopic spatial binding of the variable region of the chain, by calculating electrostatic forces, solution mediation (salvation), van der Waals gravitation and entropy values, selecting amino acids that can be optimized in the CDR1 and CDR3 sequences to reduce the affinity of HLA-DR molecules. And improve the binding of the CDR3 region of the antibody to the epitope of the epidermal growth factor receptor.

In an embodiment of the present invention, a human IgG1 heavy chain constant region Fc fragment and a light chain constant region κ fragment are respectively obtained from normal human B cells, and are ligated to the above-described heavy chain variable region and light chain variable region, respectively, to construct Antibody heavy and light chain expression vectors. The expression vector was co-transfected into mammalian cells for expression and purification to obtain humanized anti-epidermal growth factor receptor antibodies BA03, BB03, BC03 and BD03.

In an embodiment of the invention, the antibody has affinity for a cell surface epidermal growth factor receptor, blocks EGF ligand binding to a cell surface epidermal growth factor receptor, and inhibits cell surface epidermal growth factor receptor. Phosphorylation activity and ADCC action are both more active than Erbitux and have lower immunogenicity.

In an embodiment of the invention, the antibody has an inhibitory effect on the growth of various tumor cells, such as epidermal cancer, lung cancer, colon cancer, pancreatic cancer and breast cancer, and has a significant effect of inhibiting tumor growth at the animal level..

In the present invention, the scientific and technical terms used herein have the meanings commonly understood by those skilled in the art, unless otherwise stated. Also, the protein and nucleic acid chemistry, molecular biology, cell and tissue culture, microbiology, immunological related terms, and laboratory procedures used herein are terms and routine steps that are widely used in the corresponding art. Also, for a better understanding of the present invention, definitions and explanations of related terms are provided below.

In the present invention, the term "antibody" refers to an immunoglobulin molecule usually composed of two identical pairs of polypeptide chains each having one "light" (L) chain and one "heavy" (H) chain. Antibody light chains can be classified as kappa and lambda light chains. Heavy chains can be classified as μ, δ, γ, α, or ε, and the isotypes of antibodies are defined as IgM, IgD, IgG, IgA, and IgE, respectively. Within the light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, and the heavy chain further comprises a "D" region of about 3 or more amino acids. Each heavy chain consists of a heavy chain variable region (VH) and a heavy chain constant region (CH). The heavy chain constant region consists of three domains (CH1, CH2 and CH3). Each light chain consists of a light chain variable region (VL) and a light chain constant region (CL). The light chain constant region consists of one domain CL. The constant region of the antibody mediates binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (eg, effector cells) and the first component (C1q) of the classical complement system. The VH and VL regions can also be subdivided into regions with high denaturation (referred to as complementarity determining regions (CDRs)) with a more conserved region called the framework region (FR). Each VH and VL consists of three CDRs and four FRs arranged in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4 from the amino terminus to the carboxy terminus. The variable regions (VH and VL) of each

heavy/light chain pair form an antibody binding site, respectively. The assignment of amino acids to regions or domains follows the Kabat Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk (1987) J. Mol. Biol. 196:901-917; Chothia et al. (1989) Nature 342:878-883. The term "antibody" is not limited by any particular method of producing antibodies. For example, it includes, in particular, recombinant antibodies, monoclonal antibodies, and polyclonal antibodies. The antibodies may be antibodies of different isotypes, for example, IgG (eg, IgG1, IgG2, IgG3 or IgG4 subtype), IgA1, IgA2, IgD, IgE or IgM antibodies.

In the present invention, the term "antigen-binding portion" of an antibody refers to one or more portions of a full-length antibody that retain the ability to bind to the same antigen (eg, ERCC1) to which the antibody binds, competing with the intact antibody for the antigen Specific binding. See generally, Fundamental Immunology, Ch. 7 (Paul, W., ed., 2nd Ed., Raven Press, N.Y. (1989), which is incorporated herein by reference in its entirety for all purposes. The antigen binding portion can be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. In some cases, the antigen binding portion includes Fab, Fab', F(ab')2, Fd, Fv, dAb and complementarity determining region (CDR) fragments, single chain antibodies (eg, scFv), chimeric antibodies, diabody Diabody and a polypeptide comprising at least a portion of an antibody sufficient to confer specific antigen binding ability to the polypeptide.

In the present invention, the term "Fd fragment" means an antibody fragment consisting of a VH and CH1 domain; the term "Fv fragment" means an antibody fragment consisting of the VL and VH domains of a single arm of an antibody; the term "dAb" "Fragment" means an antibody fragment consisting of a VH domain (Ward et al, Nature 341:544-546 (1989)); the term "Fab fragment" means an antibody fragment consisting of VL, VH, CL and CH1 domains; The term "F(ab')2 fragment" means an antibody fragment comprising two Fab fragments joined by a disulfide bridge on the hinge region.

In some cases, the antigen binding portion of an antibody is a single chain antibody (eg, scFv), wherein the VL and VH domains form a monovalent molecule by pairing a linker that enables it to produce a single polypeptide chain (see, eg, Bird et al., Science 242:423-426 (1988) and Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988)). Such scFv molecules can have the general structure: NH2-VL-linker-VH-COOH or NH2-VH-linker-VL-COOH. Suitable prior art linkers consist of a repeating GGGGS amino acid sequence or variants thereof. For example, a linker having the amino acid sequence (GGGGS)4 can be used, but variants thereof can also be used (Holliger et al. (1993), Proc. Natl. Acad. Sci. USA 90:6444-6448). Other linkers useful in the present invention are by Alfthan et al. (1995), Protein Eng. 8:725-731, Choi et al. (2001), Eur. J. Immunol. 31:94-106, Hu et al. (1996), Cancer Res. 56:3055-3061, Kipriyanov et al. (1999), J. Mol. Biol. 293:41-56 and Roovers et al. (2001), Cancer Immunol. description.

In some cases, the antibody is a diabody, ie, a bivalent antibody, wherein the VH and VL domains are expressed

on a single polypeptide chain, but too short a linker is used to allow pairing between the two domains of the same chain, thereby forcing the domain to pair with the complementary domain of another strand and creating two antigen-binding sites (see, for example, Holliger P. Etc., Proc. Natl. Acad. Sci. USA 90:6444-6448 (1993), and Poljak R.J. Et al., Structure 2:1121-1123 (1994)).

In the present invention, the term "derived sequence" means that the sequence of the corresponding fragment of the antibody or antigen-binding portion of the present invention is highly homologous and still retains similar EGFR binding activity and immunity to the corresponding fragment of the antibody or antigen-binding portion of the present invention. The original sequence.

The antigen-binding portion of the antibody (eg, the antibody fragment described above) can be obtained from a given antibody (eg, monoclonal antibody 2E12) using conventional techniques known to those of skill in the art (eg, recombinant DNA techniques or enzymatic or chemical cleavage methods). And specifically screening the antigen-binding portion of the antibody in the same manner as for intact antibodies.

In the present invention, 20 conventional amino acids and their abbreviations follow conventional usage. See Immunology-A Synthesis (2nd Edition, E.S. Golub and D.R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991)), which is incorporated herein by reference.

In the present invention, the nucleic acid includes DNA and RNA.

Advantageous effects of the invention

The present invention is directed to the human mouse chimeric Erbituximab which has been marketed, and uses the de-immunized antibody humanization technology to change the framework region of the antibody and a part of the immunogenic CDR region to form a novel sequence of humanized resistance. Epidermal growth factor receptor IgG1 subtype antibody molecule, the obtained antibody has lower immunogenicity and higher target binding activity than erbituximab, thereby having stronger biological activity and antitumor activity in vitro and in vivo. It can be expected that the side effects of clinical application are lower and the curative effect is better.

DRAWINGS

Figure 1 shows a comparison of the affinity of antibodies to cell surface epidermal growth factor receptors.

Figure 2 shows a comparison of antibody blocking EGF ligand binding to cell surface epidermal growth factor receptor, where the ordinate is the OD 450 value.

Figure 3 shows a comparison of antibodies inhibiting epidermal growth factor receptor phosphorylation on the surface of A431 cells.

Figure 4 shows antibody-dependent cell-mediated cytotoxicity (ADCC) activity assays.

Figure 5 shows dendritic cell-T cell activation assay to determine antibody immunogenicity in vitro.

Figure 6 shows the growth inhibitory effect of antibodies on human epidermal carcinoma A431 mouse tumor model.

Detailed ways

The embodiments of the present invention will be described in detail below with reference to the accompanying drawings, however, the following examples are intended to illustrate the invention and are not intended to limit the scope of the invention. Those who do not specify the specific conditions in the examples are carried out according to the conventional conditions or the conditions recommended by the manufacturer. The reagents or instruments used are not indicated by the manufacturer, and are conventional products that can be obtained commercially.

Example 1. Design of heavy chain variable region sequence of humanized monoclonal antibody against surface epidermal growth factor receptor

The following is the sequence of the heavy chain variable region of murine anti-M225, the underlined portions are FR1, FR2, FR3 and FR4 framework sequences: QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVH WVRQSPGKGLEWLGVIWSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDTAIYYCAR ALTYYDYEFAYWGQGTLVTVSA (SEQ ID NO:17).

The human antibody gene sequence library was compared based on its heavy chain variable region FR1, FR2, FR3 and FR4 sequences to find a human germ line similar to the murine anti-M 225 heavy chain variable region FR1, FR2, FR3 and FR4 sequences (germ line) The antibody variable region corresponds to the sequence. Then, using NetMHCIIpan software or self-programming software, the affinity of the similar sequence to the HLA-DR molecule was analyzed by the in silicon method, and the frame sequence with the lowest affinity was selected, thereby finally establishing the humanized heavy chain variable region. Sequence of FR1, FR2, FR3 and FR4. Based on this framework, the spatial region of the variable region of the murine 225 antibody was analyzed using a computer molecular model, and the murine 225 antibodies required to support the CDR conformation were analyzed for the framework amino acid residues L29, L48 and L67. For the de-immunization of CDR1 and CDR3 with higher affinity for HLA-DR binding, molecular docking was used to analyze the target of human epidermal growth factor receptor M 225 antibody and its surrounding amino acid residues and humanized weight. The three-dimensional spatial combination of the variable regions of the chain, by calculating electrostatic forces, solution mediation (salvation), van der Waals gravity and entropy values (Sircar et al, SnugDock: paratope structural optimization during antibody-antigen docking compensates for errors in antibody homology models, PLoS Comput Biol. 2010 Jan 22; 6), select the amino acids that can be optimized in the CDR1 and CDR3 sequences, optimize the 33-position GLy in CDR1 to amino acid Asp, and optimize the 100-position Thr in CDR3 to amino acid Asp to reduce Affinity binding to the HLA-DR molecule and improved binding of the antibody CDR3 region to the epidermal growth factor receptor epitope. The newly designed heavy chain CDR1 is: NYDVH (SEQ ID NO:1); the newly designed heavy chain CDR3 sequence is ALDYYDYEFAY (SEQ ID NO:2) and ALTYYDYDFAY (SEQ ID NO:3), due to the low affinity of CDR2 for HLA-DR molecule binding, retains the CDR2 sequence of the original mouse anti-M 225 (the sequence is VIWSGGNTDYNTPFTS, SEQ ID NO:4). Therefore, by integrating the optimized framework sequences and CDR sequences, the four novel anti-EGF receptor humanized monoclonal antibody heavy chain variable region sequences are:

QVQLQESGPGLVKPSETLSLTCTVSGFSLSNYDVHWVRQAPGKGLEWLGVIWSGGNTDYNTPFTSRLTI SVDTSKNQFSLKLSSVTAADTAVYYCARALDYYDYEFAYWGQGTLVTVSS (SEQ ID NO:5);

QVQLQESGPGLVKPSETLSLTCTVSGFSLTNYDVHWIRQPPGKGLEWIGVIWSGGNTDYNTPFTSRVTI SVDTSKNQFSLKLSSVTAADTAVYYCARALTYYDYEFAYWGQGTLVTVSS (SEQ ID NO:6);

EVQLVESGGGLVQPGGSLRLSCAASGFSLTNYGVHWVRQAPGKGLEWLGVIWSGGNTDYNTPFTSRL TISKDNSKNTLYLQMNSLRAEDTAVYYCARALTYYDYDFAYWGQGTLVTVSS (SEQ ID NO:7);

QVQLKESGPGLVAPSQSLSITCTVSGFSLTNYGVHWVRQPPGKGLEWLGVIWSGGNTDYNTPFTSRLSI SKDNSKSQVFLRMYSLQTDDTARYYCARALTYYDYEFAYWGQGTLVTVSS (SEQ ID NO:8).

The sequence numbers of the sub-segments in the above sequences are as follows:

QVQLQESGPGLVKPSETLSLTCTVSGFSLS (SEQ ID NO:19)

QVQLQESGPGLVKPSETLSLTCTVSGFSLT (SEQ ID NO:20)

EVQLVESGGGLVQPGGSLRLSCAASGFSLT (SEQ ID NO:21)

QVQLKESGPGLVAPSQSLSITCTVSGFSLT (SEQ ID NO:22)

WVRQAPGKGLEWLG (SEQ ID NO:23)

WIRQPPGKGLEWIG (SEQ ID NO:24)

WVRQPPGKGLEWLG (SEQ ID NO:25)

RLTISVDTSKNQFSLKLSSVTAADTAVYYCAR (SEQ ID NO:26)

RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR (SEQ ID NO:27)

RLTISKDNSKNTLYLQMNSLRAEDTAVYYCAR (SEQ ID NO:28)

RLSISKDNSKSQVFLRMYSLQTDDTARYYCAR (SEQ ID NO:29)

WGQGTLVTVSS (SEQ ID NO:30)

NYGVH (SEQ ID NO:39)

ALTYYDYEFAY (SEQ ID NO:40).

Example 2. Design of light chain variable region sequence of humanized monoclonal antibody against epidermal growth factor receptor

The following is the sequence of the murine anti-M 225 light chain variable region, the underlined portions are the FR1, FR2, FR3 and FR4 framework sequences:

DILLTQSPVILSVSPGERVSFSC RASQSIGTNIH WYQQRTNGSPRLLIK YASESIS
GIPSRFSGSGSGTDFTLSINSVESEDIADYYC QQNNNWPTTFGAGTKLELK (SEQ ID NO:18).

The sequence of the light chain variable region of the murine anti-225 antibody was also analyzed, and the human antibody gene sequence library was compared according to its light chain variable region FR1, FR2, FR3 and FR4 sequences, and the murine anti-M 225 light chain variable region was found. FR1, FR2, FR3 and FR4 sequences are similar to human germline antibody variable region corresponding sequences, and then analyzed by in silicon using NetMHCIIpan software or self-programming software for affinity of selected similar sequences to HLA-DR molecules. The framework sequence with the lowest affinity was selected to finally establish the humanized sequences of FR1, FR2, FR3 and FR4 of the light chain variable region. Based on this framework, the spatial region of the variable region of the murine 225 antibody was analyzed using a computer molecular model, and the corresponding amino acid residue I58 of the murine antibody required to support the CDR conformation was analyzed. For the deimmunization of CDR3 with high affinity for HLA-DR molecule binding, molecular docking is applied to analyze the epidermal growth factor receptor mouse M 225 antibody target and its surrounding amino acid residues and humanized heavy chain. The three-dimensional spatial combination of the variable regions, by calculating electrostatic forces, solution mediation (salvation), van der Waals gravity and entropy values (Sircar et al, SnugDock: paratope structural optimization during antibody-antigen docking compensates for errors in antibody homology models, PLoS Comput Biol. 2010 Jan 22;6), select the amino acids that can be optimized in the CDR3 sequence, optimize the Asn at position 93 in the CDR3 to the amino acid Glu, and optimize the Thr at the 97 position to the amino acid Ser to reduce the binding of the HLA-DR molecule. The affinity and improve the binding of the antibody CDR3 region to the positively charged amino acid adjacent to the epidermal growth factor receptor epitope. The newly designed light chain CDR3 is: QQNNEWPTS (SEQ ID NO:9) and QQNNDWPTT (SEQ ID NO:10). Due to the low affinity of the light chain CDR1 and CDR2 for HLA-DR molecule binding, the CDR1 of the original mouse anti-M 225 (the sequence of which is RASQSIGTNIH, SEQ ID NO:11) and the CDR2 sequence (the sequence of which is YASESIS, SEQ ID NO):12). Therefore, by integrating the optimized framework sequences and CDR sequences,

the novel anti-surface epidermal growth factor receptor humanized monoclonal antibody light chain variable region sequences are:

EIVLTQSPDFQSVTPKEKVTITCRASQSIGTNIHWYQQKPDQSPKLLIKYASESISGIPSRFSGSGSGTDFTL
TINSLEAEDAATYYCQQNNEWPTSFGQGTKLEIK (SEQ ID NO:13);

EIVLTQSPDFQSVTPKEKVTITCRASQSIGTNIHWYQQKPDQSPKLLIKYASESISGIPSRFSGSGSGTDFTL
TINSLEAEDAATYYCQQNNNWPTTFGAGTKLEIK (SEQ ID NO:14)

DIVLTQSPATLSVTPGESVSFSCRASQSIGTNIHWYQQKSHESPRLLIKYASESISGIPSRFSGSGSGTDFTLSI
NSVETEDFGMYYCQQNNNWPTTFGAGTKLEIK (SEQ ID NO:15)

EIVLTQSPDFQSVTPKEKVTITCRASQSIGTNIHWYQQKPDQSPKLLIKYASESISGIPSRFSGSGSGTDFTL
TINSLEAEDAATYYCQQNNDWPTTFGQGTKLEIK (SEQ ID NO:16).

The sequence numbers of the sub-segments in the above sequences are as follows:

EIVLTQSPDFQSVTPKEKVTITC (SEQ ID NO:31)

DIVLTQSPATLSVTPGESVSFSC (SEQ ID NO:32)

WYQQKPDQSPKLLIK (SEQ ID NO:33)

WYQQKSHESPRLLIK (SEQ ID NO:34)

GIPSRFSGSGSGTDFTLTINSLEAEDAATYYC (SEQ ID NO:35)

GIPSRFSGSGSGTDFTLSINSVETEDFGMYYC (SEQ ID NO:36)

FGQGTKLEIK (SEQ ID NO:37)

FGAGTKLEIK (SEQ ID NO:38)

QQNNNWPTT (SEQ ID NO:41).

The humanized antibodies obtained according to the above examples have the following combinations:

Table 1. Humanized anti-EGFR antibody combination

[image]

Example 3 Preparation of Humanized Monoclonal Antibody Against Epidermal Growth Factor Receptor

(1) According to the antibody heavy chain variable region sequence and the light chain variable region sequence determined in Examples 1 and 2, PCR primer oligonucleotide fragments encoding heavy chain and light chain variable region sequences were designed and synthesized, respectively, and introduced during synthesis. The enzyme cleavage site. PCR primer oligonucleotide fragments are typically about 54 bases in length and about 18 bases overlap in adjacent oligonucleotide fragments. The same amount of each PCR primer fragment was mixed to carry out an overlap extension PCR reaction.

PCR reaction system: dNTPs 0.2 μM (final concentration); 1 μl of each PCR primer fragment; 10 μ buffer 3 μl; cloned pfu (Invitrogen) 1 μl; water was added to 30 μl.

PCR reaction conditions: 94° C 3 min → (94° C 30 s → 56° C 30 s → 72° C 1 min) × 30 → 72° C 10 min

The PCR product was separated by 1% agarose gel electrophoresis, and the purified fragment was recovered. After EcoR I digestion, the antibody heavy chain variable region sequence and the light chain variable region sequence were cloned into pCR-Blunt II-TOPO (Invitrogen) vector. After transformation into E. coli TOP10 (Invitrogen), screening was performed on LB/Kanamycin plates, and 10 white plaques were inoculated in LB

liquid medium containing Kanamycin, and QIquick PCR purification kit was used. Plasmids were extracted and sequenced to determine the heavy and light chain variable region sequences.

(2) Construction of expression vector

RNA was isolated from normal human B cells, and the human IgG1 heavy chain constant region IgG1 Fc fragment and the light chain constant region κ fragment were obtained by RT-PCR, and the desired cleavage site was introduced at RT-PCR, and pre-constructed into pcDNA3. In the expression vector of.1 (Invitrogen), pcDNA3.1-Fc and pcDNA3.1-κ expression vectors were obtained, and positive clones were identified by DH5 bacterial transformation, plasmid extraction and sequencing.

The sequence of the human IgG1 heavy chain constant region IgG1 Fc fragment is:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV
PSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV
TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS
NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:42)

The sequence of the light chain constant region kappa fragment is:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS
TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:43)

The heavy chain variable region of the antibody was excised from the positive clone of pCR-BluntII-TOPO by Eco47III/NheI, and the clone was ligated into the pcDNA3.1-Fc expression vector, and the heavy chain variable region was ligated to the constant region. The light chain variable region of the antibody was excised from the positive clone of pCR-BluntII-TOPO by AscI/BsiWI, and the clone was ligated into the pcDNA3.1-κ expression vector to link the variable region of the light chain to the constant region (Reference can be found in Morrison). SL. Cloning, expression, and modification of antibody V regions. Curr Protoc Immunol. 2002 May; Chapter 2: Unit 2.12.). Positive clones were again determined by DH5 bacterial transformation, plasmid extraction and sequencing. The sequencing results were identical to the encoded sequences of the recorded antibodies, i.e., expression vectors for the heavy and light chains of the antibody, respectively.

(3)Transfection of CHO cells and expression of specific antibodies

Cell lines and culture conditions: CHO-S cells (purchased from Invitrogen) were cultured under conditions of 1×CD-CHO (purchased from GIBCO), 1×HT (purchased from GIBCO), 8 mM glutamine (purchased from GIBCO), and cultured in 37° C, 8% CO 2 incubator. The antibody heavy chain expression vector obtained by the step (2) and the light chain expression vector were co-transfected into CHO-S cells, and DMRIE-C transfection kit (purchased from Invitrogen) was used, and the instructions were operated according to the kit instructions. On the third day after transfection, cells were cultured in the above culture medium and subjected to pressure screening by adding 500 μg/ml of G418 (purchased from GIBCO) and 12.5 μg/ml of puromycin (purchased from Sigma). After about 14 days of pressurization, the selected positive polyclonal was cultured in a six-well plate, and the specific antibody expression amount was detected by a direct ELISA method (coated with EGFR-Fc from the expressed 293T cells). The positive polyclonal with the highest expression rate was selected. After 7 days of large-scale culture, the culture supernatant was collected by centrifugation, purified by affinity chromatography column (purchased from GE) of protein A, dialyzed to PBS, and filtered through a 0.22 um membrane. The purified antibodies BA03, BB03, BC03 and BD03 were obtained separately for the following examples.

Example 4 Flow cytometry was used to detect the affinity of antibodies to cell surface epidermal growth factor receptor

The specific affinity of the expressed antibody for the epidermal growth factor receptor was detected by A431 cells (ATCC). A431 cells were combined with different concentrations of product antibodies at 4° C for 1 hour. The cells were washed three times with PBS, and 100-fold diluted FITC-labeled anti-human Fcγ-specific secondary antibody (purchased from The Jakson Laboratory) was added at 4° C for one hour. The combined fluorescent signal was detected by flow cytometry after washing three times with PBS (Fig. 1). The EC50 (half effective concentration) value was calculated using the Prism software based on the average fluorescence intensity, and the results are shown in Table 2. The results showed that the humanized antibodies BA03 and BD03 of the present invention have higher binding activity than Erbitux (purchased from Baitai Biopharmaceutical Co., Ltd.), and BB03 and BC03 are similar to Erbitux. The binding activity of all four antibodies was stronger than that of Nimotuzumab (purchased from Merck KgaA).

Table 2 EC50 values of antibody-EGFR binding activity

Example 5 Antibody Blocks EGF Binding to Cell Surface Epidermal Growth Factor Receptor

The blocking ability of the expressed antibody to bind EGF to the cell surface epidermal growth factor receptor was examined using the A431 cell (ATCC) Cell-ELISA method (see Bioo Scientific MaxDiscovery in Cell ELISA kit instructions). Different concentrations of antibodies were incubated with A431 cells for 1 hour at 4° C, and biotinylated recombinant human epidermal growth factor EGF (purchased from R&D) was added and washed three times with PBS. Biotin-labeled recombinant human epidermal growth factor binding to the cell surface epidermal growth factor receptor was then detected with HRP-labeled avidin (Fig. 2). The IC50 (half inhibitory concentration) value was calculated using Prism software based on the measured OD 450 value, and the results are shown in Table 3. The results indicate that the humanized antibodies BA03 and BD03 of the present invention have stronger blocking activity of ligand-cell surface epidermal growth factor receptor binding than erbitux, and BB03 and BC03 are similar to Erbitux. The blocking activity of the four antibodies was stronger than that of Taixinsheng.

Table 3 IC50 values of antibodies blocking the binding activity of EGF to EGFR

Example 6 Antibody inhibits surface epidermal growth factor receptor phosphorylation of A431 cells

5×10<5> cells/well A431 cells were seeded with 6-well plates and cultured overnight with complete medium (DMEM + 10% FBS). The cells were cultured in 2 ml DMEM to make the cells lack nutrients for 16-18 hours. Then, 1 ml of pre-warmed fresh medium (DMEM, without 10% FBS) was used for 1-2 hours. Five ug/ml of anti-epidermal growth factor receptor antibody was added to A431 cells 60 min before stimulation with 50 ng/ml recombinant EGF (epidermal growth factor, purchased from R&D systems). Wash 3 times with pre-cooled 1×PBS, add 250 μl of cell lysate (cell lysis buffer, purchased from Cell Signalling), lyse, centrifuge, 14000 rpm, 4° C, 15 minutes, collect the lysate, quantify with BCA kit protein.. Epidermal growth factor receptor phosphorylation was determined by western blot using anti-pEGFR (pY1068) antibody (purchased from Abnova), and total epidermal growth factor receptor content was determined by anti-total epidermal growth factor receptor antibody (purchased from Abnova).. The results indicate that the humanized antibody BA03 of the present invention has a stronger inhibitory activity against surface epidermal growth factor receptor phosphorylation of A431 cells than Erbitux. Both are stronger than TaiXinsheng (Figure 3).

Example 7 In vitro antibody-dependent cell-mediated cytotoxicity (ADCC) assay

The blood obtained from normal volunteers was inhaled into a heparinized syringe and mixed with HBSS (Life Technologies) in the same volume without Ca<2+>/Mg<2+> and placed on a lymphocyte gradient separating agent (Life Technologies). The layers were stratified and centrifuged at 800 g for 30 minutes. PBMCs collected on the interface were washed in HEPES buffered saline and resuspended in assay medium containing

1% heat inactivated FBS (HyClone Laboratories, Logan, UT), 2 mM L-glutamine, 10 mM HEPES, And 50 mg/mL gentamicin in RPMI 1640 medium (Life Technologies). Ramos cells (ATCC) (10<4> cells/well) and various concentrations of antibodies (disposed in 50 mL of assay buffer) placed in 50 mL of assay buffer were added to round-bottom 96-well plates. This mixture was pre incubated for 30 minutes at 37° C. 50 μL of 5×10<5> effector cells were then dispensed into each well and incubated for 4 h at 37° C. Experiments were performed using a 40:1 effector cell (PBMC):target cell (Ramos cell) ratio. The plate was centrifuged at 250 g for 10 minutes, and then the supernatant was collected. The lactic acid dehydrogenase activity in the supernatant was determined using a cytotoxicity test kit (Boehringer Mannheim, Indianapolis, IN) and the manufacturer's instructions, and the percentage of toxicity was calculated from the average absorbance of the sample. Specific lysis rate = (average experimental release - average spontaneous release) / (average maximum release - average spontaneous release). As a result, as shown in Fig. 4, the humanized antibodies BA03 and BD03 of the present invention have higher ADCC activity than cetuximab, and are higher than Erbitux at 0.1 ug/ml and 1 ug/ml ($p<0.05$, Student's t test), BB03 and BC03 are similar to Erbitux.

Example 8 Dendritic cell-T cell activation assay to determine antibody immunogenicity in vitro

Blood obtained from normal volunteers (n=12) was inhaled into a heparinized syringe and mixed with HBSS (Life Technologies) containing no Ca2<+>/Mg2<+> in the same volume, placed in a lymphocyte gradient separating agent. (Life Technologies) layered and centrifuged at 800g for 30 minutes. Peripheral blood mononuclear cells collected on the interface were washed in HEPES buffered saline and resuspended in assay medium RPMI 1640 medium (Life Technologies). The resulting peripheral blood mononuclear cells were continuously cultured for 2 hours in 50 ml of a 1:100 dilution of beta-mercaptoethanol serum-free AIM V medium (Gibco) to harvest monocytes. 800 units/ml of GM-CSF (Endogen) and 500 units/ml of IL-4 (Endogen) were added; incubation was continued for 5 days. Then, 0.2 units/ml of TNFα (Endogen) and IL-1α (Endogen) at a final concentration of 50 units/ml were further added for 2 days. On day 7, 50 mg/ml of Mitomycin C was added for 1 hour to stop the continued differentiation of dendritic cells, and then the differentiated mature dendritic cells were collected by centrifugation at 600G. Inoculate a round bottom 96-well culture plate at 2 x 10<4> cells/well (100 ul). Negative selection of CD4<+> Cellect Kit (Biotex) was used to collect human CD4<+> T cells in AIM V medium containing 2% human serum. In a 96-well culture plate containing 100 μl of dendritic cells (1 × 10<4> cells/ml), 10 μl of a 5 mM test antibody (control group added with DMSO) was added, and then 100 μl of CD4<+> was added to each well. T cells (1 × 10<5> cells/ml). The number of activated T cell proliferation was determined using the alamar blue reagent (for the assay method, refer to Invitrogen's alamara Blue Assay kit kit instructions). The results were converted to T cell activation index (calculated as T cell activation index = T cell proliferation number of antibody group / T cell proliferation number of DMSO group, reference can be made to Stickler MM et al. A human dendritic cell-based method to identify CD4+T-cell epitopes in potential protein allergens.

Environ Health Perspect. 2003 February; 111 (2): 251-254.). As a result, as shown in FIG. 5, the four humanized

antibodies of the present invention have lower immunogenicity than the human and mouse chimeric antibodies erbitux, and BA03 and BB03 are significantly lower than those of ipituto ($p<0.05$)., Student's t test).

Example 9 In vitro antitumor activity of BA03 against lung cancer, colon cancer, pancreatic cancer and breast cancer tumor cells

The in vitro anti-tumor activity of BA03 on lung cancer, colon cancer, pancreatic cancer and breast cancer tumor cells was investigated for ADCC activity and growth inhibition in vitro. The ADCC activity measurement method was the same as in Example 7. In vitro growth inhibition was determined as follows: 300-2000 cells/well of different tumor cells were seeded in 96-well plates (the cell density was determined according to the characteristics of different tumor cells), and BA03 and IgG1 were added to the complete medium at a final concentration of 10 μg/ml. After 5-10 days of culture of the control antibody (purchased from R&D systems), cell viability was measured by adding Alamablue (the assay method is described in the Invitrogen alamara Blue Assay kit kit). Relative cell specific activity (%ADCC) and cell growth inhibition rate were calculated in comparison with IgG1. Each tumor cell line growth inhibition test was repeated at least twice (three replicate wells). After adding the antibody to the tumor cells, PE fluorescent-labeled secondary antibody (PE-labled anti-human IgG1, Southern biotechnology) was added, and the expression level of EGFR was determined by FACS. As a result, as shown in Table 4, the humanized antibody BA03 of the present invention significantly inhibited the growth of various lung cancer, colon cancer, pancreatic cancer and breast cancer tumor cells (Table 4).

Table 4: In vitro activity of BA03 on different tumor cells

[image]

[image]

Inhibition rate: +/-5-10%, +10-20%, ++20-40%

Example 10 Animal Pharmacodynamics Experiment

Balb/c nude mice were injected subcutaneously on day 0 with human skin basal cell carcinoma A431 cells (5×10^{6} cells/0.1 ml per mouse). The size of the tumor was measured with a caliper, and the size of the tumor was calculated using the formula (length x width^{2})/2. When the tumor size averaged 100 mm^{3} (61-158 mm

< 3 >), tumor-bearing mice were randomly assigned to 3 groups according to tumor size. Each Monday and Thursday in each week was administered at 5 mg/kg in the peritoneal cavity for a total of 5 doses. Saline (as a negative control). The volume of the tumor is shown in Fig. 6. In the saline control group, the tumor rapidly increased to > 1500 mm < 3 > in three weeks. The growth and control of the mouse tumor after the humanized antibody BA03 and the erbitux treatment of the present invention were compared. The group was significantly inhibited ($p < 0.001$, Student's t test), but the humanized BA03 antibody had better antitumor activity than Erbitux ($p < 0.05$, Student's t test).

Although specific embodiments of the invention have been described in detail, those skilled in the art will understand. Various modifications and alterations of the details are possible in light of the teachings of the invention. The full scope of the invention is given by the appended claims and any equivalents thereof.



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Biodegradable polymeric micelles composed of doxorubicin conjugated PLGA–PEG block copolymer

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Abstract

Biodegradable polymeric micelles containing doxorubicin in the core region were prepared from a di-block copolymer composed of doxorubicin-conjugated poly(DL-lactic-co-glycolic acid) (PLGA) and polyethyleneglycol (PEG). The di-block copolymer of PLGA–PEG was first synthesized and the primary amino group of doxorubicin was then conjugated to the terminal hydroxyl group of PLGA, which had been pre-activated using *p*-nitrophenyl chloroformate. The resulting polymeric micelles in aqueous solution were characterized by measurement of size, drug loading, and critical micelle concentration. The micelles containing chemically-conjugated doxorubicin exhibited a more sustained release profile than PEG–PLGA micelles containing physically-entrapped doxorubicin. The cytotoxic activity of the micelles against HepG2 cells was greater than free doxorubicin, suggesting that the micelles containing conjugated doxorubicin were more effectively taken up cellularly, by an endocytosis mechanism rather than by passive diffusion. Confocal microscopic observation and flow cytometry analysis supported the enhanced cellular uptake of the micelles. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Doxorubicin; Biodegradable; Micelles; PLGA; Endocytosis

1. Introduction

Amphiphatic block copolymers self-assemble into polymeric micelles in aqueous solution, and potentially can be used as parenteral drug delivery systems [1–5]. Various polymeric micelles have been used for the solubilization of water insoluble drugs within the interior region of micelles. The size of polymeric micelles, approximately 50 nm, not only makes them ideal drug delivery carriers for escaping from renal exclusion and the reticulo-endothelial system, but also enhances their vascular permeability [2]. A few studies of polymeric micelles containing anti-cancer drugs have proposed the possibility of passively targeting solid tumors, compared to the extensive research on drug–polymer conjugates and liposomes [6,7]. For instance, di-block copolymers composed of poly(aspartic acid)–poly(ethyleneglycol) have been used to physically entrap or chemically conjugate doxorubicin within the polyanionic core of micelles, and have resulted in an effective suppression of tumor growth when injected in vivo. Intravenously-injected micelles diffused through loose-

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ly packed endothelial cell junctions of blood capillaries in the vicinity of tumor tissue and then accumulated in the solid tumor by 'enhanced permeation and retention' (EPR) effect [2]. Moreover, enhanced uptake of polymeric drugs within tumor cells was believed to occur by increased endocytotic transport and by circumventing the multi drug resistance (MDR) effect [7,8].

Most polymeric micelles are composed of hydrophobic segments as the internal core and hydrophilic segments as a surrounding corona [9]. The hydrophobic core serves as a reservoir for hydrophobic drugs, whereas the hydrophilic shell affects their pharmacokinetic behavior. In particular, biodegradable polymeric micelles have received much attention. Various aliphatic polyesters such as poly(L-lactic acid) (PLA), poly(DL-lactic-co-glycolic acid) (PLGA), and poly(ϵ-caprolactone) (PCL) have been combined with a hydrophilic PEG segment to produce an A–B type di-block copolymer structure. For example, di-block copolymers of PLA–PEG and PCL–PEG have been synthesized by ring opening polymerization of L-lactide and ϵ-caprolactone, respectively, in the presence of methoxy-terminated PEG [10,11]. These polymeric micelles, however, have a relatively low loading of moderately water-soluble drugs due to a limited partitioning of more hydrophilic drugs into the hydrophobic polymer phase. This can be regarded as one of the major limitations of polymeric micelle systems.

In this paper, we describe a doxorubicin delivery system using a di-block copolymer composed of doxorubicin conjugated PLGA and PEG. It was expected that a greater amount of doxorubicin could be loaded into the inner PLGA core of these polymeric micelles, relative to the physical entrapment of free doxorubicin. A mixture of DL-lactide and glycolide, molar ratio of 50/50, was ring opening polymerized in the presence of monomethoxy PEG 2000. The terminal hydroxyl group of the PLGA chain end was utilized to conjugate doxorubicin after activation with *p*-nitrophenyl chloroformate. The polymeric micelles were characterized and their cytotoxicity against HepG2 cells was investigated. Confocal microscopy and flow cytometry studies were also carried out to elucidate the transport of these micelles within cells.

2. Experimental methods

2.1. Materials

DL-lactide and glycolide, purchased from Purac (Gorinchem, The Netherlands), were re-crystallized from ethyl acetate before use. Monomethoxy poly(ethyleneglycol) (mPEG), M_w 2000, stannous octoate, doxorubicin, *p*-nitrophenyl chloroformate, and 3-(4,5-dimethylthiaol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma (St. Louis, MO). Human hepatoblastoma cell line (HepG2) was obtained from Korean Cell Line Bank (KCLB). All other chemicals were of analytical grade.

2.2. Preparation of doxorubicin conjugated PLGA–PEG block copolymer

DL-lactide (14.4 g), glycolide (11.6 g), and mPEG (8 g) in a bottle-neck flask were heated to 140°C under nitrogen atmosphere for complete melting. The molar ratio of DL-lactide and glycolide was 1:1. Then, 0.05% (w/w) stannous octoate was added, and the temperature of the reaction mixture was raised to 180°C and maintained for 3 h. The polymerization was carried out under vacuum. The polymer was recovered by dissolution in methylene chloride followed by precipitation in ice-cold diethyl ether [10]. The precipitate was filtered and dried under reduced pressure. To PLGA–PEG di-block copolymer (3 g) dissolved in methylene chloride (30 ml), dropwise *p*-nitrophenyl chloroformate (80 mg) and pyridine (63 mg) were added; (PLGA–PEG–*p*-nitrophenyl chloroformate–pyridine, stoichiometric molar ratio: 1/2/4) at 0°C. After 3 h at room temperature under nitrogen, the activated PLGA–PEG di-block copolymer was recovered by precipitation in ice-cold diethyl ether. The precipitate was filtered and dried under reduced pressure. The activated PLGA–PEG (0.25 g) in dimethylformamide (7.5 ml) was reacted with doxorubicin (10 mg) in the presence of triethylamine (6.75 mg) for 24 h at room temperature under nitrogen (stoichiometric molar ratio of activated PLGA–PEG–doxorubicin–triethylamine: 1/1/4). The doxorubicin–PLGA-b–PEG (DOX–PLGA–PEG) was purified as polymeric micelles by adding

the organic phase to deionized water, followed by dialyzing three times against deionized water for 4 h (Spectra/Por 7, MWCO 10 000). After the dialysis, DOX–PLGA–PEG was freeze dried and stored at −20°C. The doxorubicin content was determined to be 36.5 mol% by measuring the UV absorbance of a DMSO solution at 480 nm. A calibration curve was constructed using different concentrations of free doxorubicin in DMSO.

2.3. Preparation of doxorubicin loaded micelles

DOX–PLGA–PEG (100 mg) in acetone (10 ml) was added to PBS buffer (100 ml, pH 7.4). Spontaneous formation of micelles was confirmed by the observation of a transparent reddish solution. For the preparation of PLGA–PEG micelles containing physically entrapped doxorubicin, PLGA–PEG (97.8 mg) and doxorubicin (2.2 mg) co-dissolved in acetone (10 ml) were added to PBS buffer (100 ml). The resulting micelles contained a large fraction of unentrapped doxorubicin, detected by reversed phase HPLC. The doxorubicin-loaded micelles were extensively dialyzed to remove unentrapped doxorubicin before further use.

2.4. Size distribution and doxorubicin loading amount and efficiency

To confirm the formation of micelles, size distribution was measured using a laser light scattering technique (ZetaPlus, Brookhaven Instrument Corp., USA). The loading amount and loading efficiency were calculated based on the weight ratio of the loaded amount of doxorubicin to the amount of micelles, and the percent ratio of the amount of doxorubicin incorporated into the micelles to an initial amount used, respectively [12]. The doxorubicin-loaded micelles were freeze-dried, dissolved in DMSO, and then used for the determination of doxorubicin in the micelles as described above.

2.5. Determination of critical micelle concentration (CMC) of DOX–PLGA–PEG

The CMCs of the doxorubicin conjugated di-block copolymer and unconjugated di-block copolymer were determined using pyrene as an extrinsic probe [13]. Briefly, pyrene (4 μg) pre-dissolved in methylene chloride (10 ml), was added to a test tube and the solvent was evaporated. Deionized water (5 ml) was added to the test tube and different amounts of doxorubicin-conjugated and unconjugated di-block copolymers dissolved in acetone was added to the aqueous phase. The solution was incubated at room temperature with mild stirring for complete evaporation of the organic solvent to induce the formation of micelles. The fluorescence intensity of the solution was measured at room temperature using a spectrofluorometer (Shimadzu RF5301PC, Japan). The concentration of pyrene was fixed at 0.2 μM and the final concentration of DOX–PLGA–PEG was varied from 10 to 1000 μM. Emission spectra were obtained at 377, 383 and 392 nm, which were determined from the scanning emission spectrum of each sample from 300 to 600 nm, fixing the excitation wavelength at 339 nm. The CMC was determined by taking a mid-point of the copolymer concentration at which the relative emission fluorescence intensity ratio measured at 383 nm to 377 nm was varied. Since the emission wavelength of doxorubicin appearing around 530 nm was far from those of pyrene, the CMC values could be determined without interference from the presence of the conjugated doxorubicin within the micelles.

2.6. Drug release experiment

Twenty mg of micelles in PBS buffer (20 ml) were sealed in a dialysis bag (M_w cutoff: 10 000, Spectra/por), and incubated in PBS buffer (30 ml) at 37°C. The doxorubicin released into the incubation medium was collected at pre-determined time intervals and stored frozen for quantitative analysis. The release amount was determined from the absorbance at 480 nm.

2.7. Determination of DOX–PLGA micelles transported into HepG2 cells

HepG2 cells were used as a model cancer cell line for the examination of endocytosis. HepG2 cells, 5×10^3 cells/ml, were seeded onto a 100 mm culture

dish or cover glass pre-soaked in culture medium and after 24 h DOX–PLGA–PEG micelles or free doxorubicin were added to the culture medium, both of which had the same doxorubicin concentration of 170 μM. The loading amount of doxorubicin in DOX–PLGA–PEG micelles was taken into account to adjust the doxorubicin concentration. After 6 h, the cells on the cover glass were thoroughly washed three times with PBS and examined by a confocal microscopy (Carl Zeiss LSM5100, Germany) at an excitation wavelength of 488 nm. An emitting signal was detected at 515 nm. In order to quantify the amount of doxorubicin within the cell, the cells were harvested and analyzed by flow cytometry (Becton Dickinson FACSCalibur, USA), and the gate was arbitrary set for the detection of green fluorescence (150<FL1-H<400, 0<FSC<200, linear scale). The relative fluorescence intensity of HepG2 cells incubated with DOX–PLGA–PEG micelles was calculated using those incubated with free doxorubicin as a control, 100%. The reason for using such a high concentration of doxorubicin in this experiment, although it was high enough for inducing cell death, was to clearly visualize the confocal microscopic image and to readily observe the FACS results. The exposure time, 6 h, was sufficient to observe the endocytic behavior of the micelles within HepG2 cells, but it was too short to kill the HepG2 cells. Cell death was observed by a trypan blue exclusion method.

2.8. Cytotoxicity assay

Cytotoxicities of doxorubicin-loaded micelles were determined against the human hepatoblastoma cell line (HepG2). Free doxorubicin and the doxorubicin-loaded micelles were used for determining the inhibition of cell growth employing a tetrazolium dye (MTT) assay. HepG2 cells harvested in a logarithmic growth phase were seeded on 96 wells at a cell density of 5×10^3 cells/ml. Growth medium DMEM was used and a humidified atmosphere (5% CO_2) was maintained. After 72 h incubation, the MTT assay was performed as a function of doxorubicin concentrations and the percentage of cell viability was determined.

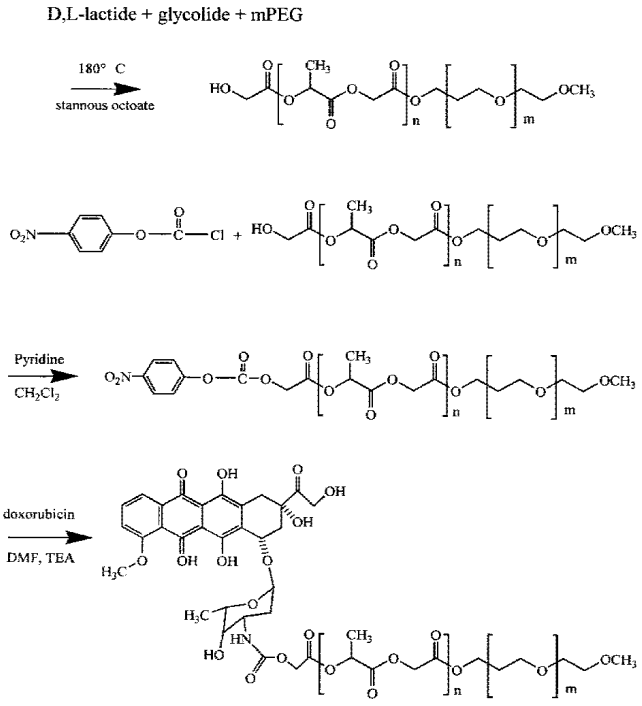


Fig. 1. Synthetic route to DOX–PLGA–PEG conjugate.

3. Results and discussion

The synthesis of DOX–PLGA–PEG is shown in Fig. 1. A di-block copolymer of PLGA–PEG was first prepared by ring opening polymerization of DL-lactide and glycolide in the presence of mPEG (M_w 2000) [10]. The M_n of the copolymer and the molar ratio of DL-lactide to glycolide were determined by ^{1}H NMR to be 9600 and 50:50, respectively. The M_n, M_w and polydispersity were determined by GPC to be 13 000, 23 000 and 1.7, respectively. GPC showed a single peak that was eluted earlier than those of PLGA and PEG, confirming the successful synthesis of the PLGA–PEG di-block copolymer and the absence of PLGA and PEG homopolymers. The T_g was determined by DSC to be 1.2°C.

Doxorubicin was conjugated to PLGA via a carbamate linkage between the primary amine group in doxorubicin and the terminal hydroxyl group in PLGA, activating the latter with *p*-nitrophenyl chloroformate. The conjugation of doxorubicin to PLGA–PEG was confirmed by the GPC elution

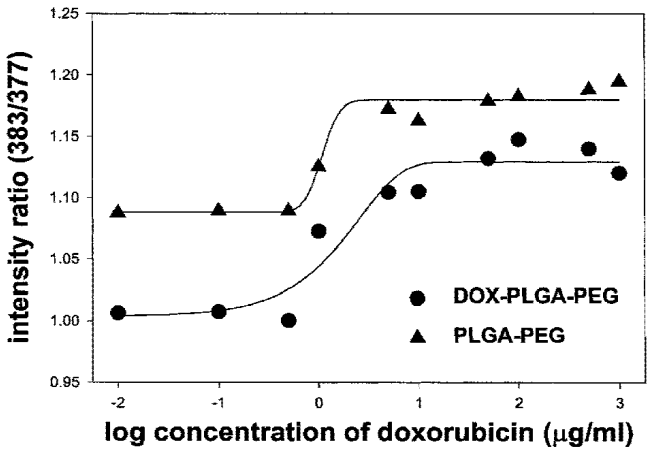


Fig. 2. Critical micelle concentration of DOX–PLGA–PEG and PLGA–PEG.

profiles of the PLGA–PEG and DOX–PLGA–PEG, which were monitored by refractive index and fluorescence detectors, respectively. A single peak was eluted at the same retention time using both refractive index and fluorescence detectors, without free PEG and doxorubicin peaks. This indicated that DOX–PLGA–PEG was successfully purified by dialysis after the conjugation. However, it should be mentioned that the sample consisted of 36.5 mol% DOX–PLGA–PEG and 63.5 mol% PLGA–PEG.

The CMC of DOX–PLGA–PEG was determined using pyrene as an extrinsic probe according to the previously published method [13]. As shown in Fig. 2, the CMC of DOX–PLGA–PEG micelles was approximately 0.1 μg/ml, similar to the CMC of doxorubicin unconjugated PLGA–PEG micelles. Thus, the conjugation of doxorubicin to PLGA–PEG did not affect the micelle-forming ability of PLGA–PEG. This can be attributed to the relatively small molecular size of doxorubicin compared to the size of the hydrophobic PLGA chain segment in the block copolymer. This result also suggests that moderately hydrophobic doxorubicin, conjugated to the terminal end of PLGA chain, may be located in the hydrophobic core of the micelles without disturbing the micellar structure.

The physical characteristics of DOX–PLGA–PEG micelles, specifically the effective diameter, and the doxorubicin loading amount and efficiency, are compared in Table 1 with those of PLGA–PEG micelles that were physically loaded with free doxorubicin. The sizes of DOX–PLGA–PEG and PLGA–PEG micelles, as determined by a dynamic laser scattering method, were both about 60 nm in diameter. This finding is consistent with the CMC result that doxorubicin conjugation to PLGA did not effect the micelle-forming capability. The slightly increased diameter (61.5 nm) of DOX–PLGA–PEG, relative to the diameter (58.2 nm) of PLGA–PEG, may be attributed to doxorubicin conjugation. The loading amount and efficiency of free doxorubicin within polymeric PLGA–PEG micelles were 0.51% (w/w) and 23.2%, respectively. On the other hand, those of the DOX–PLGA–PEG micelles were 2.18% (w/w) and 99.1%, respectively. The enhanced loading amount and efficiency for the micelles prepared from DOX–PLGA–PEG were clearly attributable to the fact that doxorubicin conjugated to PLGA–PEG was forcefully located within the core of hydrophobic PLGA chains. In the case of physical entrapment, the moderately water-soluble doxorubicin can escape into the aqueous phase during the self-assembling process. The partition behavior of doxorubicin between the water phase and the PLGA bulk phase will determine the loading amount and efficiency. The limitation of drug loading efficiency within polymeric micelles can be overcome by the conjugation approach.

The release of doxorubicin from DOX–PLGA–

Table 1
Effective diameters and loading efficiency of doxorubicin polymeric micelles

Doxorubicin loading	Effective diameter (nm)	Theoretical loading (w/w, %)	Actual loading (w/w, %)	Loading efficiency (%)
Physically entrapped (PLGA–PEG)	58.21±6.21	2.20	0.51±0.07	23.18±3.18
Conjugated (DOX–PLGA–PEG)	61.48±7.17	2.20	2.18±0.04	99.09±1.81

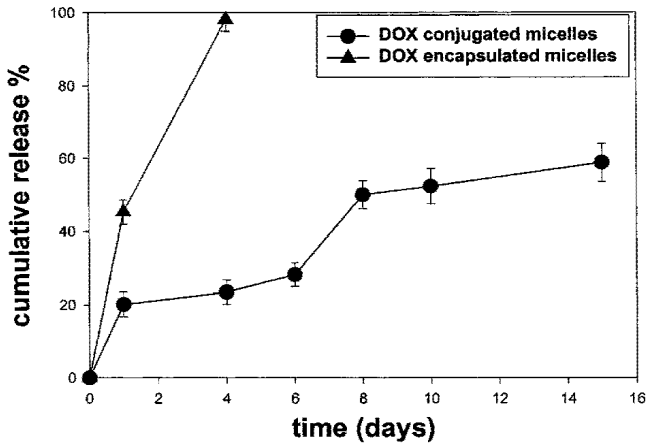


Fig. 3. Release profiles of doxorubicin from polymeric micelles. DOX conjugated micelles were composed of DOX–PLGA–PEG and DOX encapsulated micelles were composed of PLGA–PEG having physically entrapped doxorubicin.

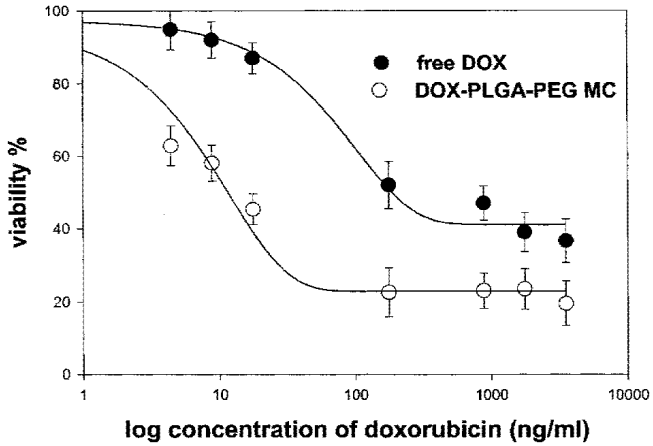


Fig. 4. In vitro cytotoxicities of doxorubicin polymeric micelles and free doxorubicin against HepG2 cells.

PEG micelles and PLGA–PEG micelles containing physically entrapped doxorubicin is shown in Fig. 3. While the DOX–PLGA–PEG micelles showed sustained release of up to 50% of the drug load over 2 weeks, release from PLGA–PEG micelles containing non-conjugated doxorubicin lasted only for 3 days. The sustained release of DOX–PLGA–PEG micelles is attributed to the gradual hydrolysis of the conjugated PLGA backbone and subsequent controlled liberation of water-soluble DOX–PLGA oligomer conjugates in the incubation medium [14]. The sustained release of doxorubicin from the DOX–PLGA–PEG micelles was similarly observed in formulations of PLGA microspheres and nanoparticles that were prepared by the conjugation of various hydrophilic drugs to PLGA [12,15,16]. Since the carbamate linkage between doxorubicin and PLGA chain is hydrolyzed very slowly, the released doxorubicin fraction contains a mixture of doxorubicin–PLGA oligomer species. Consequently, the doxorubicin released from the micelles may have slightly modified properties. This concern was discussed in the previous reports [15]. However, it was found that the released doxorubicin fraction from doxorubicin-conjugated PLGA nanoparticles exhibited a comparable in vitro cyototoxic effect to free doxorubicin against HepG2 cells [15]. Thus, it was of interest to study whether the DOX–PLGA–PEG micelles had a comparable cytotoxic effect.

The cytotoxicities of free doxorubicin and DOX–PLGA–PEG micelles against HepG2 cells are compared as shown in Fig. 4. In this experiment, freshly prepared DOX–PLGA–PEG micelles were directly used to test the in vitro cytotoxicity. For comparison, the doxorubicin concentration in DOX–PLGA–PEG was adjusted to be the same as that of free doxorubicin. It can be seen that DOX–PLGA–PEG micelles are about 10-fold more cytotoxic than free doxorubicin. This remarkable result can be explained by the enhanced uptake of doxorubicin conjugated PLGA–PEG micelles relative to free doxorubicin. The uptake of DOX–PLGA–PEG micelles by HepG2 cells was investigated qualitatively and quantitatively using a confocal microscopy and a flow cytometry, respectively. As shown in Fig. 5, HepG2 cells incubated with DOX–PLGA–PEG micelles emitted more intense fluorescence than those incubated with free doxorubicin. The enhanced uptake of DOX–PLGA–PEG micelles can be attributed to their facilitated endocytotic transport, relative to passive diffusion of free doxorubicin through the cell membrane. It is likely that the increased uptake of micelles occurred by a 'fluid-phase endocytotic mechanism' because there is no specific affinity between the outer PEG chains of the micelles and negatively charged plasma membrane of the cells. Additionally, the multi-drug resistance (MDR) effect might play a role in reducing the accumulation of free doxorubicin within cells: free doxorubicin molecules transported within cells are likely to be pumped

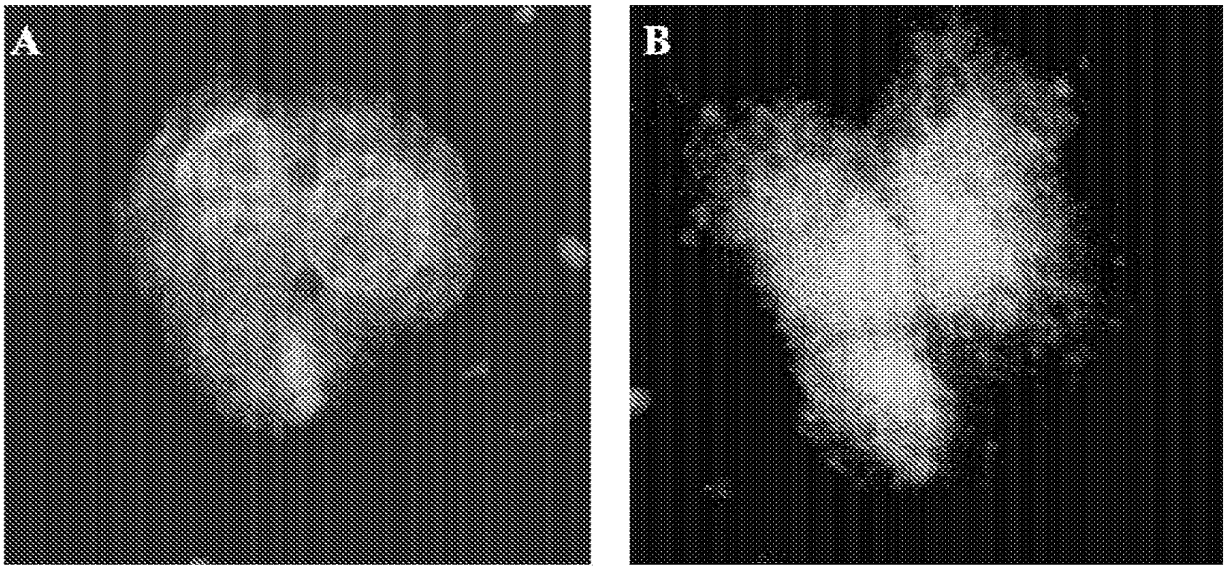


Fig. 5. Confocal microscopy of HepG2 cells containing doxorubicin polymeric micelles. (A) HepG2 cells incubated with free doxorubicin and (B) HepG2 cells incubated with DOX–PLGA–PEG micelles.

back out of the cytosol by the *p*-glycoprotein pump expressed in the membrane of cancerous cells. From this standpoint, cellular delivery of doxorubicin based on polymeric micelles is an attractive way to circumvent the MDR effect. In the previous study of PLGA nanoparticles formulated with PLGA conjugated doxorubicin, a similar enhanced uptake of doxorubicin by HepG2 cells was observed [12,16]. While HepG2 cells incubated with free doxorubicin emitted fluorescence mainly at the nucleus, those with DOX–PLGA–PEG micelles showed some fluorescence in the cytosol as well as intense fluorescence at the nucleus (Fig. 5).

Flow cytometry also confirmed the enhanced uptake of DOX–PLGA–PEG micelles. As shown in Fig. 6, HepG2 cells incubated with DOX–PLGA–

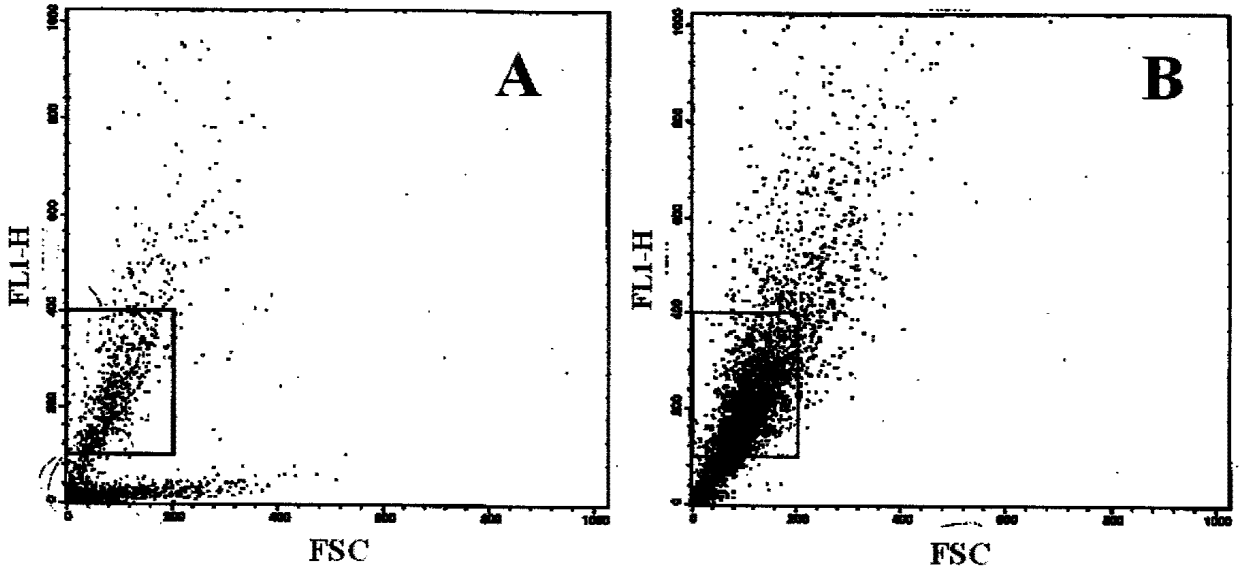


Fig. 6. Dot plots of flow cytometry analysis, (A) free doxorubicin and (B) DOX–PLGA–PEG micelles. Cell count was 5×10^3.

PEG micelles emitted higher fluorescent intensity than those with free doxorubicin. When the fluorescence intensities of the two samples within the arbitrary set gated region (150<FL1-H<400, 0< FSC<200) were compared, HepG2 cells incubated with DOX–PLGA–PEG micelles showed five times more fluorescence intensity than those with free doxorubicin in the selected region. Thus, it can be said that DOX–PLGA–PEG micelles are taken up by HepG2 cells to a much greater extent than free doxorubicin.

4. Conclusions

A higher loading of doxorubicin in polymeric micelles could be obtained by conjugation of doxorubicin to a di-block copolymer of PLGA–PEG. There was no change in critical micelle concentrations of PLGA–PEG with and without conjugation to doxorubicin. While DOX–PLGA–PEG micelles showed a sustained release profile over 2 weeks, PLGA–PEG micelles containing physically entrapped doxorubicin exhibited a burst release at the initial stage of incubation. DOX–PLGA–PEG micelles were more cytotoxic against HepG2 cells than free doxorubicin. This was ascribed to the greater uptake of DOX–PLGA–PEG micelles by HepG2 cells, compared to free doxorubicin, resulting from enhanced endocytosis of DOX–PLGA–PEG micelles.

Acknowledgements

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References

[1] K. Kataoka, G. Kwon, M. Yokoyama, T. Okano, Y. Sakurai, Block copolymer micelles as vehicles for drug delivery, J. Controlled Release 24 (1992) 119–132.

[2] G.S. Kwon, T. Okano, Polymeric micelle as new drug carriers, Adv. Drug Del. Rev. 16 (1996) 107–116.

[3] G. Kwon, M. Naito, M. Yokoyama, T. Okano, Y. Sakurai, K. Kataoka, Block copolymer micelles for drug delivery: loading and release of doxorubicin, J. Controlled Release 48 (1997) 195–201.

[4] G.S. Kwon, S. Suwa, M. Yokoyama, T. Okano, Y. Sakurai, K. Kataoka, Physical entrapment of adriamycin in AB block copolymer micelles, Pharm. Res. 12 (1995) 192–195.

[5] M. Yokoyama, G.S. Kwon, M. Naito, T. Okano, Y. Sakurai, K. Kataoka, Preparation of micelle-forming polymer–drug conjugates, Bioconjug. Chem. 3 (1992) 295–301.

[6] R. Duncan, Drug–polymer conjugates: potential for improved chemotherapy, Anti-cancer Drugs 3 (1992) 175–210.

[7] T. Minko, P. Kopeckova, V. Pozharov, J. Kopecek, HPMA copolymer bound adriamycin overcomes MDR1 gene encoded resistance in a human ovarian carcinoma cell line, J. Controlled Release 54 (1998) 223–233.

[8] A. Colin de Verdiere, C. Dubernet, F. Nemati, M.F. Poupon, F. Puisieux, P. Couvreur, Uptake of doxorubicin from loaded nanoparticles in multidrug resistant leukemic murine cells, Cancer Chemother. Pharmacol. 33 (1994) 504–508.

[9] M. Malmsten, B. Lindman, Self-assembly in aqueous block copolymer solution, Macromolecules 25 (1992) 5440–5445.

[10] J.H. Jeong, D.W. Lim, D.K. Han, T.G. Park, Synthesis, characterization and protein adsorption behaviors of PLGA/PEG di-block co-polymer blend films, Colloid. Surf. B: Biointerfaces 18 (2000) 371–379.

[11] M. Yuan, Y. Wang, X. Li, C. Xiong, X. Deng, Polymerization of lactides and lactones. 10. Synthesis, characterization, and application of amino-terminated poly(ethylene glycol)–co-poly([e]-caprolactone) block copolymer, Macromolecules 33 (2000) 1613–1617.

[12] H.S. Yoo, J.E. Oh, K.H. Lee, T.G. Park, Biodegradable Nanoparticles Containing Doxorubicin–PLGA Conjugates for Sustained Release, Pharm. Res. 16 (1999) 1114–1118.

[13] C. Zhao, M. Winnik, G. Reiss, M. Croucher, Fluorescence probe technique used to study micelle formation in water-soluble block copolymers, Langmuir 6 (1990) 514–516.

[14] T.G. Park, Degradation of poly(DL-lactic) microsphere: effect of molecular weight, J. Controlled Release 30 (1994) 161–173.

[15] J.E. Oh, Y.S. Nam, K.H. Lee, T.G. Park, Conjugation of drug to poly(DL-lactic-co-glycolic acid) for controlled release from biodegradable microspheres, J. Controlled Release 57 (1999) 269–280.

[16] H.S. Yoo, T.G. Park, In vitro and in vivo anti-tumor activities of nanoparticles based on doxorubicin–PLGA conjugates, J. Controlled Release 68 (2000) 419–431.

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Meeting Report

Cancer Research

Challenges and Key Considerations of the Enhanced Permeability and Retention Effect for Nanomedicine Drug Delivery in Oncology

Uma Prabhakar[1], Hiroshi Maeda[2], Rakesh K. Jain[3], Eva M. Sevick-Muraca[5], William Zamboni[6], Omid C. Farokhzad[4], Simon T. Barry[7], Alberto Gabizon[8], Piotr Grodzinski[1], and David C. Blakey[7]

Abstract

Enhanced permeability of the tumor vasculature allows macromolecules to enter the tumor interstitial space, whereas the suppressed lymphatic filtration allows them to stay there. This phenomenon, enhanced permeability and retention (EPR), has been the basis of nanotechnology platforms to deliver drugs to tumors. However, progress in developing effective drugs using this approach has been hampered by heterogeneity of EPR effect in different tumors and limited experimental data from patients on effectiveness of this mechanism as related to enhanced drug accumulation. This report summarizes the workshop discussions on key issues of the EPR effect and major gaps that need to be addressed to effectively advance nanoparticle-based drug delivery. *Cancer Res; 73(8); 2412–7. ©2013 AACR.*

Introduction

The field of nanomedicine, despite being conceptualized as far back as the 1980s, is only now transitioning in a broad sense from academic research to drug development and commercialization. In oncology, unique structural features of many solid tumors, including hypervasculature, defective vascular architecture, and impaired lymphatic drainage leading to the well-characterized enhanced permeability and retention (EPR; ref. 1) effect, are key factors in advancing this platform technology. However, the EPR effect has been measured mostly, if not exclusively, in implanted tumors with limited data on EPR in metastatic lesions. Dextran-coated iron oxide nanoparticles (25–50 nm) have been used clinically for several years (2) to measure permeability and retention noninvasively by MRI (3). Furthermore, tumor response alone is no longer considered a good endpoint, at least from the health authority point of view. This is exemplified by the recent U.S. Food and Drug Administration (FDA) withdrawal of bevacizumab (Avastin) for patients with metastatic breast cancer where impressive tumor responses were seen but bevacizumab showed no improvement in overall survival. Thus, limitations and challenges both in understanding tumor structural features and correlating them with the technology must be addressed and additional critical data need to be generated before nanotechnology-based drug delivery approaches can be fully realized in clinical use in patients with cancer. A one-day workshop was convened at the NIH on October 10, 2012, to specifically address key issues related to understanding of EPR effect and its use to achieve the maximum therapeutic effect with drugs using nanoparticle carriers.

This workshop was organized by the Alliance for Nanotechnology in Cancer and its recently formed public–private partnership consortium, TONIC (Translation of Nanotechnology in Cancer), in response to several questions raised by industry members of TONIC. The main purpose of this meeting was to gain better understanding of the EPR characteristics impacting the use of nanoparticles in the clinic. Experimental evidence of EPR in animal models and humans, clinical relevance of EPR, gaps in knowledge, and ways to address these gaps were all discussed.

Report

The workshop composed of 8 talks covering topics ranging from methods to investigate EPR in preclinical and clinical studies including diagnostic imaging, to the ramifications of EPR for enhanced drug uptake by different tumors and the predictability of preclinical and clinical outcomes. The session opened with an overview of the nanotechnology programs in cancer, funded by the Alliance for Nanotechnology in Cancer (NCI), and was followed by an introduction to TONIC, a corporate partnership model of the public, private, and

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Note: A list of speakers is available as supplementary data for this article at Cancer Research Online (http://cancerres.aacrjournals.org/).

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academic sectors, to accelerate the translation and development of nanotechnology solutions for the early detection, diagnosis, and treatment of cancer. This was followed by scientific presentations relating to the key questions identified at previous TONIC meetings. The discussions at the workshop focused on two key themes, namely, heterogeneity of EPR in tumors and factors that influence EPR effect.

Heterogeneity of EPR in Tumors

EPR exists in tumors and can be exploited for selective delivery of drugs to tumor by nanotechnology. However, there is significant heterogeneity within and between tumor types. It was noted that different tumor types have different pore dimensions in the vasculature and that the maximum pore size changes with the location for a given type of tumor (i.e., primary vs. metastases). In addition, there may be differences in vessel structure within a single tumor type. Thus, to understand whether a tumor is likely to respond to a nanoparticle-based drug that relies on EPR for delivery, an image-guided patient selection or diagnostic approach can potentially prove useful to profile and select tumor types and patients with tumors conducive to such delivery. Hiroshi Maeda (Sojo University, Kumamoto, Japan), who first proposed the EPR effect over 25 years ago (1), suggested a number of ways one can augment the EPR effect. These included increasing the blood pressure during infusion of a nanomedicine or macromolecular drug using angiotensin-II (e.g., blood pressure increase from 100 → 150 mmHg). Other methods involve vascular mediators such as nitroglycerin, ACE inhibitor, or PGE1 agonist (beraprost) and these have been shown to be effective in *in vivo* tumor models resulting in better tumor delivery (2- to 3-fold increase), linked to improved therapeutic effect (4).

Factors Influencing EPR

The following factors influence the EPR effect in tumors: (i) the nature of both the vascular bed and surrounding stroma, the presence or absence of functional lymphatics and interstitial hydraulic conductivity impacting interstitial pressure along with mechanical stresses generated by cancer and stromal cells impacting the extracellular matrix; (ii) tumor size, type, and location (including primary tumor versus metastatic lesions); (iii) extent of macrophage tumor infiltration and the activity of the mononuclear phagocytic system (MPS), which can vary between and within tumor types plus patient characteristics (e.g., age, gender, tumor type, body composition, treatment). These factors lead to accumulation of nanoparticles in both normal tissues and in different sections of the tumor, for example, in the periphery, viable tumor, and necrotic sections; and (iv) co-medications, which may impact, among other things, stroma and blood pressure (hypertension increases tumor blood flow). In addition, several vascular factors (Table 1; ref. 4), such as nitric oxide generators (5) and bradykinin potentiators, that is, ACE inhibitors that lower blood pressure, are known to affect EPR and are relatively safe and inexpensive to combine with a nanoparticle drug (4).

A fundamental limitation in evaluating EPR and the factors that affect EPR is poor understanding of which preclinical tumor models recapitulate patients with solid tumors. The factors affecting delivery of nanoparticles to tumors in preclinical models, such as tumor growth environment, vasculature, functional MPS, etc., appear to vary based on the cancer model [e.g., syngeneic flank xenograft, orthotopic xenograft, genetically engineered mouse model (GEMM)]. Thus, future studies will need to systemically evaluate these factors in preclinical models and in patients with various solid tumors and determine whether the models represent all aspects of the EPR effect.

The observed heterogeneity in EPR may be a contributing factor to the limited impact of nanoparticle-based drugs with reductions in toxicity and gains in overall survival as compared with small-molecule anti-cancer agents. Table 2 summarizes objective data on the survival benefits from nanotherapeutics approved to date. Further understanding and predictability of EPR function in primary tumor and its metastatic sites through the use of imaging studies may aid the development of future, effective nanodrugs. Correlation of EPR activity to clinical responses would likely provide direct clinical data to determine whether tumors with high EPR tumor activity will be more amenable to effective treatment using nanoparticle-based therapies (5). It was noted that the diversity of nanoparticle characteristics and API used is expected to impact the applicability of such correlations across different nanoparticle platforms and products.

The optimal patient selection or diagnostic aid to measure the EPR activity within a patient needs to be further defined. Ideally, this would involve a single imaging agent that is generalizable to all nanoparticles. Given the heterogeneity of nanoparticle-based systems—size, shape, charge characteristics, etc.—a specific diagnostic agent might, however, be required to predict likely response to a particular nanoparticle relying on EPR delivery. The use of contrast agents and MRI to measure the enhanced permeability (EP) component of the EPR effect might be one generic method. Others might include a defined nanoparticle of a fixed size (~100 nm) labeled with an appropriate imaging agent—for example, Cu^{64} for positron emission tomography (PET) or fluorescent marker for near-infrared fluorescence (NIRF). There is precedence for a range of labeled liposomes and iron oxide–loaded nanoparticles for imaging, but there are very few human clinical studies on nanoparticle imaging that can effectively address the prevalence of EPR. In one such study, the biodistribution and pharmacokinetics of $^{[111]}$In-labeled PEGylated liposomes was evaluated in patients with locally advanced cancers. Positive tumor images were obtained in 15 of 17 studies, although levels of tumor liposome uptake varied between and within tumor types (6).

Eva Sevick-Muraca (The University of Texas Health Science Center, Houston, Texas) discussed the use of NIRF to image lymphatic flow and with fluorescent agents to detect cancers. This technique is light based and the fluorescent dye has no half-life and can be repeatedly excited, making it more appropriate for imaging of nanoparticle accumulation over longer timeframes than radioactive imaging agents with short half-lives (7). While NIRF is considered to be a combination product by the FDA and has a maximum tissue penetration of 3 to 5 cm,

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Table 1. Factors affecting the EPR effect of macromolecular drugs in solid tumors (modified after references 4 and 5)

Mediators	Responsible enzymes and mechanisms	Possible application to therapeutic modality and mechanism
Bradykinin	Kallikrein/protease	ACE inhibitors (e.g., enalapril); blocking of kinin degradation elevates local kinin level → more EPR.
NO	iNOS	NO-releasing agents (e.g., nitroglycerin, ISDN, etc.) via denitrase and nitrite reductase to generate NO.
VPF/VEGF	Involved in NO generation	
Prostaglandins	COX-1	Beraprost sodium: PGI_2 agonist works via vascular dilatation and extravasation (5).
Collagenase (MMP)	Activated from proMMPs by peroxynitrite, or proteases	
Peroxynitrite	$NO + O_2$	
Carbon monoxide (CO)	Heme oxygenase (HO)-1	PEG-hemine via induction of HO-1 in tumor → CO generation (15).
Induced hypertension	Using angiotensin II	Slow i.v. infusion → systemic hypertension, vascular extravasation selectively in tumor tissue.
Inflammatory cells and H_2O_2	Neutrophil/NADPH oxidase, etc.	
TGF-β inhibitor		Inducing multiple inflammatory cytokines; NOS, COX, etc.: NO, PGs, etc.
TNF-α		Inducing multiple inflammatory cytokines; NOS, COX, etc.: NO, PGs, etc.
Anticancer agents		
Heat	Vascular dilation	Gold nanoparticle or ferrite nanoparticle using electromagnetic, or laser, or microwave.

such devices are not yet available in hospitals and may not have the right sensitivity at this time to detect the marker agent. The ability to image lymphatic function in the tumor vicinity could also provide a means to assess interstitial pressure imbalances. Efforts are underway to include dual-labeling PET for presurgical imaging and then NIR guidance during surgery (8). It is anticipated that PET will remain a crucial tool for clinical imaging and that the optical imaging counterpart will add value rather than being a replacement.

Ways to enhance the EPR effect in tumors were discussed and included drugs that impacted the vasculature (4)–for example, VEGF-based antagonists leading to vessel normalization, agents causing hypertension and increasing tumor blood flow, and agents that modulate the tumor matrix. Agents that generate nitric oxide [nitroglycerine or ISDN (isosorbide dinitrate)] were also shown to be effective in humans (4, 5). ACE inhibitor (e.g., enarapril), which potentiates the action of bradykinin, is also effective (4). Further work is required to validate the benefits of such agents in the context of exploiting the enhancement of EPR effect in the clinical setting (4, 5). It was suggested that both optimization of the nanoparticle and optimization of the tumor microenvironment were required for optimal delivery. Rakesh K. Jain (Harvard Medical School, Boston, Massachusetts); hypothesized that normalizing the vasculature, extracellular matrix, and lymphatics will lead to better delivery of drugs (9). However, normalized vasculature means that the average pore is smaller and this may require the use of smaller nanoparticles (~20 nm particle size). Overall, the biologic impact of the abovementioned vascular effectors on delivery of nanoparticles of varying composition, shape, and flexibility needs significant further work.

The role of the lymphatics in tumor biology and nanoparticle delivery was discussed. This highlighted the need to consider changes in physiologic status, both in the acute and in long-term functionality of lymphatics in patients with cancer influenced by inflammation, tumor burden, or treatment. This is an area of active research and imaging techniques are being developed that will allow this to be explored in more detail.

In terms of animal tumor models to evaluate the EPR effect, subcutaneous flank tumor xenografts were thought to offer limited value. The vasculature of such models often resembles the vasculature found in very high EPR tumors, for example, renal tumors irrespective of tumor type, and thus probably gives a false impression about the benefit of nanoparticle-based drugs relying on the EPR effect in most tumor settings. The workshop participants felt that better options are provided by metastatic, orthotopic, and GEMM-based models, although these need further characterization and validation. Primary tumor explants may be another option to model delivery to tumor types with high stromal content. Further work is required to understand how to use the preclinical tumor models to investigate drugs relying on the EPR effect for activity and to understand how they reflect the heterogeneity seen in clinical disease. The site of the tumor was also considered to be important, and a more systematic assessment of vasculature architecture versus site of tumor was recommended.

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Table 2. Survival benefits from the FDA-approved nanomedicines to date

Generic drug	Trade name(s)	Indication	Benefit
PEGylated liposomal doxorubicin	Doxil and Caelyx	HIV-related Kaposi's sarcoma	No statistically significant change in overall survival (23 wks) vs. doxorubicin, bleomycin, and vincristine treatment (22.3 wks) for HIV-related Kaposi's sarcoma
		Metastatic ovarian cancer	Statistically significant overall survival improvement (108 wks, $P = 0.008$) vs. topotecan treatment (71.1 wks) for platinum-sensitive patients with ovarian cancer
		Metastatic breast cancer	No statistically significant overall survival change (84 wks) vs. conventional doxorubicin (88 wks) for patients with breast cancer receiving first-line therapy
Liposomal daunorubicin	DaunoXome	HIV-related Kaposi's sarcoma	No statistically significant overall survival change (52.7 wks) vs. doxorubicin, bleomycin, vincristine treatment (48.9 wks)
Poly (styren-*co*-maleic acid)–conjugated naocarzinostatin	SMANCS	Liver cancer, renal cancer	Approved in 1993 in Japan. Far more effective when the EPR is enhanced by increasing the blood pressure in difficult-to-treat tumors, including metastatic liver cancer, cancers of pancreas, gall bladder, etc.
			Liver cancer: 5-year survival (%)**
			Metastasis: 1 seg.+ > 2 seg.
			Child A: >90% ~ >50%
			Child B: 40% 30%
			Five-year survival (%) based on the liver function (cirrhosis) by child classification and intrahepatic+ metastasis within one segment or more
Albumin-bound paclitaxel	Abraxane	Metastatic breast cancer	Statistically significant overall survival change (56.4 wks, $P = 0.024$) vs. polyethoxylated castor oil–based paclitaxel treatment (46.7 wks) for patients receiving second-line treatment

NOTE: The polymeric platform methoxy PEG-poly(D,L-lactide) taxol with the trade name Genexol-PM (Sanayang Co.) has been approved in Korea for the treatment of metastatic breast cancer. Adapted from the work of Jain and Stylianopoulos (16).
**, SMANCS data in the table were provided by H. Maeda.

Omid Farokhzad (Harvard Medical School) discussed the advantages of including a targeting agent on the nanoparticle to enhance the retention component and/or enable delivery of drug directly into the tumor cell via internalization of the nanoparticle. The majority of the currently available clinical data on nanoparticle oncology drugs relate to passively targeted liposomal drugs. Recently, several actively targeted nanoparticle products have also entered clinical development, including liposomes and polymeric particles containing payloads ranging from conventional cytotoxic drugs to genes expressing tumor suppressors (10). These particles are targeted to various tumor markers including the transferrin receptor HER-2 and prostate-specific membrane antigen (PSMA) using either protein or small-molecule ligands. Recent data were presented for BIND-014 (11), a docetaxel-encapsulated polymeric nanoparticle targeted to PSMA, which is expressed on the surface of prostate cancer cells and nonprostate solid tumor neovasculature. In preclinical studies, BIND-014 increased the concentration of docetaxel in PSMA-expressing solid tumor xenografts by 5- to 10-fold. In a phase I clinical trial in patients with advanced solid tumors, BIND-014 displayed signals of antitumor efficacy in patients with advanced and metastatic cancer at low doses and in tumors where conventional docetaxel has minimal activity. With progress in polymeric nanoparticle engineering, similar approaches are also being applied to existing and developmental anticancer drugs, including other cytotoxics and molecularly targeted agents such as kinase inhibitors, and it will only be a matter of time before these advances will ultimately impact the treatment of cancer.

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William Zamboni (University of North Carolina, Chapel Hill, North Carolina) characterized the pharmacologic properties of nanoparticles *in vivo* as part of preclinical and clinical studies. He stressed the importance of the MPS, tissue distribution, and potential tumor delivery on the clearance of nanoparticles. There is a bidirectional interaction between monocytes and liposomal agents and potentially other nanoparticle agents (12, 13). Monocytes internalize liposomes, which then releases the drug from the liposome and leads to toxic effects to the monocytes. The tissue distribution and tumor delivery of nanoparticles may involve MPS-mediated and non–MPS-mediated mechanisms where uptake of nanoparticles by circulating MPS cells compared with tumoral macrophages may result in different tumor drug exposure and responses. Dr. Zamboni has developed an ex *vivo* flow cytometry–based, high-throughput screening platform (HTSP) system called PhenoGLO-HTSP to measure the clearance of nanoparticles by the MPS and bidirectional interaction between the MPS and nanoparticles, conjugates, and antibody–drug conjugates. Importantly, this method also predicts nanoparticle pharmacokinetics and pharmacodynamics in humans where the MPS system seems to drive the clearance, efficacy, and toxicity of nanoparticle agents. PhenoGLO-IT can measure MPS function in a blood sample from patients as a method to individualize the dose of nanoparticle agents and/or as a biomarker for predicting pharmacokinetics and pharmacodynamics (response and toxicity) of nanoparticles.

The workshop participants felt that as our understanding of nanoparticle delivery to tumors increases, the emerging nanoformulations should be considered both as a general formulation strategy in drug development and as a selected strategy to improve delivery profiles of existing or failed drugs.

Prospects

During discussions at the conclusion of the symposium, participants recommended the formation of a working group to establish translational and clinical procedures for integrated clinical trials involving nanotherapeutic constructs and accompanying imaging approaches. Such translational studies and clinical trials would enable further understanding and predictability of EPR function in a tumor and its primary or metastatic sites and may be critical for the development of future effective nanodrugs and predictive of antitumor response (14). An additional recommendation from this workshop was to generate a position paper highlighting key translational studies that should be conducted and parameters that should be monitored in nanoparticle drug delivery clinical trials to enable testing of various hypotheses for effective nanoparticle delivery (tumor perfusion, vascular permeability, interstitial penetration, retention, lymphatic function, MPS activity, blood pressure, fluid and solid stresses, others). In coming months, symposium participants will actively pursue these key recommendations and develop the necessary tools required to advance the scientific translation of the nanotechnology platform in the oncology therapeutic area.

Disclosure of Potential Conflicts of Interest

D.C. Blakey has ownership interest (including patents) in AstraZeneca. R.K. Jain is employed (other than primary affiliation; e.g., consulting) as a cofounder and board member of XTuit Pharmaceuticals, and as a board member of Hambrecht & Quist Healthcare Investors and Hambrecht & Quist Life Sciences Investors; has a commercial research grants from MedImmune, Roche, and Dyax; has ownership interest (including patents) in XTUit Pharmaceuticals; and is a consultant/advisory board member of Enlight Biosciences, SynDevRx, Dyax, Noxxon Pharmaceuticals, and Zyngenia. E.M. Sevick-Muraca has ownership interest (including patents) in NIRFImaging, Inc. W. Zamboni has ownership interest (including patents) in PhenoGLO Technologies. O.C. Farokhzad has ownership interest (including patents) in and is a consultant/advisory board member of BIND Bioscience, Selecta Bioscience, and BLEND. A. Gabizon has a commercial research grant from Janssen Pharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

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References

1. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy - Mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. Cancer Res 1986;46:6387–92.
2. Harisinghani MG, Barentsz J, Hahn PF, Deserno WM, Tabatabaei S, van de Kaa CH, et al. Noninvasive detection of clinically occult lymph-node metastases in prostate cancer. N Engl J Med 2003; 19:2491–9.
3. Gaglia JL, Guimaraes AR, Harisinghani M, Turvey SE, Jackson R, Benoist C, et al. Noninvasive imaging of pancreatic islet inflammation in type 1A diabetes patients. J Clin Invest 2011;121: 442–5.
4. Maeda H, Nakamura H, Fang J. The EPR effect for macromolecular drug delivery to solid tumors: improved tumor uptake, less systemic toxicity, and improved tumor imaging ĐReview of the vascular permeability of tumors and the EPR effect. Adv Drug Deliver Rev 2013;65:71–9.
5. Maeda H. Macromolecular therapeutics in cancer treatment: the EPR effect and beyond. J Control Release 2012;164:138–44.
6. Harrington KJ, Mohammadtaghi S, Uster PS, Glass D, Peters AM, Vile RG, et al. Effective targeting of solid tumors in patients with locally advanced cancers by radiolabeled pegylated liposomes. Clin Cancer Res 2001;7:243–54.
7. Sevick-Muraca EM. "Translation of near-infrared fluorescence imaging technologies: emerging clinical applications." Annu Rev Med 2012; 63:217–31.
8. Hall MA, Pinkston KL, Wilganowski N, Robinson H, Ghosh P, Azhdarinia A, et al. "Comparison of mAbs targeting EpCAM for detection of prostate cancer lymph node metastases with multimodal contrast: quantitative uPET/CT and NIRF imaging." J Nucl Med 2012;53: 1497–37.
9. Jain RK. Normalizing tumor microenvironment to treat cancer: Bench to bedside to biomarkers. J Clin Oncol. In press, 2013.

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10. Kamaly N, Xiao Z, Valencia PM, Radovic-Moreno AF, Farokhzad OC. Targeted polymeric therapeutic nanoparticles: design, development and clinical translation. Chem Soc Rev 2012;41:2971–3010.
11. Hrkach J, Von Hoff D, Mukkaram Ali M, Andrianova E, Auer J, Campbell T, et al. Preclinical development and clinical translation of a PSMA-targeted docetaxel nanoparticle with a differentiated pharmacological profile. Sci Transl Med 2012;4:128ra39.
12. Caron WP, Rawal S, Song G, Kumar P, Lay JC, Zamboni WC. Bidirectional interaction between nanoparticles and cells of the mononuclear phagocyte system. In: Dobrovolskaia MA, McNeil SE, editors. Immunological properties of Engineered Nanomaterials. Singapore: World Scientific Publishing Co.; 2012.
13. Caron WP, Song G, Kumar P, Rawal S, Zamboni WC. Pharmacokinetic and pharmacodynamic disposition of carrier-mediated agents. Clin Pharmacol Ther 2012;91:802–12.
14. Petersen AL, Hansen AE, Gabizon A, Andresen TL. Liposome imaging agents in personalized medicine. Adv Drug Deliv Rev 2012;64: 1417–35.
15. Fang J, Qin H, Nakamura H, Tsukigawa K, Maeda H. Carbon monoxide, generated by heme oxygenase-1, mediates the enhanced permeability and retention (EPR) effect of solid tumor. Cancer Sci 2012;102:535–41.
16. Jain RK, Stylianopoulos T. Delivering nanomedicine to solid tumors. Nat Rev Clin Oncol 2010;7:653.

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