SUMMARY

[0013a] In an aspect of the present disclosure, there is provided a use of an antibody-drug conjugate (ADC) in the preparation of a medicament for treating urothelial carcinoma, wherein the antibody-drug conjugate is an anti-HER2 antibody or a functional fragment thereof coupled to a cytotoxic molecule,

wherein the antibody-drug conjugate is represented by formula Ab-(L-U)n, wherein Ab is the antibody or the functional fragment thereof, L is a linker, U is a conjugated cytotoxic molecule, and n is an integer from 1 to 8, representing the number of therapeutic agent molecules bound to the antibody,

wherein the antibody or the functional fragment thereof is capable of competing with a defined antibody against the same epitope,

wherein the defined antibody comprises a heavy chain variable region and a light chain variable region defined by Complementarity Determining Region (CDR) sequences, and wherein

(i) the heavy chain variable region comprises three CDRs, wherein the amino acid sequences of the CDRs are respectively set forth in SEQ ID NO: 1, 2 and 3; and

(ii) the light chain variable region comprises three CDRs, wherein the amino acid sequences of the CDRs are respectively set forth in SEQ ID NO: 4, 5 and 6; and

wherein L is Maleimido-Caproyl-valine citrulline p-amino-benzyloxy (mc-vc-pAB), and

U is MMAE.

[0017] The present disclosure provides a method for treating urothelial carcinoma. The method comprises injecting an effective amount of an antibody-drug conjugate (ADC) into a patient, wherein the ADC is an anti-HER2 antibody conjugated cytotoxic molecule. The cytotoxic molecule includes, but is not limited to, a tubulin inhibitor or a DNA damaging agent. The tubulin inhibitor includes, but is not limited to, dolastatin and derivatives thereof, auristatin and derivatives thereof, and maytansine and derivatives thereof; the DNA damaging agents include, but are not limited to calicheamicins, duocarmycins, anthramycin derivative PBD (pyrrolobenzodiazepine), and camptothecin derivative SN-38. The auristatin and derivatives thereof include, but are not limited

to, monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF), auristatin F (AF) or derivatives thereof; the maytansine and derivatives thereof include, but are not limited to, DM1, DM3, DM4 and derivatives thereof (Research progress of bullet molecules of antibody-drug conjugates, Hu Xinyue et al., Chin Med Biotechnol, December 2017, Vol. 12, No. 6) (Research progress of maytansinoid antibody drug conjugates, Zhou Lei et al., Chinese Journal of New Drugs, Volume 25, Issue 22, 2521 -2530). The cytotoxic molecules may also be amanitins, anthracyclines, baccatins, camptothecins, cemadotins, colchicines, colcimids, combretastatins, cryptophycins, discodermolides, docetaxel, doxorubicin, echinomycins, eleutherobins, epothilones, estramustines, lexitropsins, maytansines, methotrexate, netropsins, puromycins, rhizoxins, taxanes, tubulysins, or vinca alkaloids. The cytotoxic molecules are not limited to the above-mentioned categories, and may include all drugs that can be used for ADC.

[0018] Another ~~aspect~~example of the present disclosure is to provide an antibody-drug conjugate (ADC) for use in the manufacture of a medicament for treating bladder cancer. The antibody-drug conjugate comprises an antibody or a functional fragment thereof capable of binding to HER2, wherein the antibody comprises a heavy chain variable region and a light chain variable region, and wherein (i) the heavy chain variable region comprises three CDRs, wherein the CDRs have amino acid sequences of SEQ ID NO : 1, 2 and 3, respectively; and (ii) the light chain variable region comprises three CDRs, wherein the CDRs have the amino acid sequences of SEQ ID NO: 4, 5 and 6, respectively. The antibody may also be an antibody capable of competing with a

defined antibody against the same or similar epitope, wherein the defined antibody comprises the above CDRs.

[0019] In the present disclosure, the term "antibody" may include a full-length antibody or an antibody fragment that binds to, reacts with, or composites with HER2. An antibody can be any protein, protein-like molecule, or polypeptide that binds, composites, or reacts with a portion of a population of cells seeking therapeutic modification. The antibody may be a chimeric antibody or a functionally active fragment thereof, a humanized antibody or a functionally active fragment thereof, a human antibody or a functionally active fragment thereof. It can also be an antibody or a functionally active fragment thereof of derives from another species other than the above species, for example: mouse antibody or functionally active fragment thereof, rat antibody or functionally active fragment thereof, goat antibody or functionally active fragment thereof, rabbit antibody or functionally active fragment thereof. The antibody may be a polyclonal antibody or a monoclonal antibody. In some ~~embodiment~~examples, the antibody may be a bispecific antibody. Also, the antibody may be a functionally active fragment, a derivative or analog of the antibody. "Functionally/functional" means that the fragments, derivatives, or analogs can recognize the same antigen, and the antibodies that can recognize fragments, derivatives, or analogs derived from the antigen, such as but not limited to: F (ab ')$_2$, Fab, Fab ', Fv fragments and dimers of antibody heavy chains and light chains, or any minimal fragments thereof, like Fvs or single chain antibodies (SCAs). In addition, the antibody may be a fusion protein of an antibody. Antibodies may also include analogs and derivatives that are modified or unmodified (i.e., covalently linked to any molecule), as long as such covalent bonding allows the antibody to retain its antigen-binding immunospecificity. Examples include, but are not limited to, analogs and derivatives of antibodies, including further modifications such as: glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization via known protecting / blocking groups, protease cleavage, attachment to cellular antibody units or other proteins, and the like. Any bulk chemical modifications can be achieved using known techniques, including but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis in the presence of tunicamycin, and the like. In addition, analogs or derivatives may include one or more unnatural amino acids. In some ~~embodiment~~examples, the antibody may have modifications (e.g., substitutions, deletions, or additions) in amino acid residues that interact with the Fc receptor. In another ~~aspect~~example,

the antibody-drug conjugate has a structure represented by formula Ab-(L-U)n, wherein Ab is the antibody or a functional fragment thereof, L is a linker, U is a coupled cytotoxic molecule, n is an integer from 1 to 8, representing the number of therapeutic agent molecules bound to the antibody.

[0020] In another ~~aspect~~example, the linker is linked to the antibody or the functional fragment thereof via a thiol group and/or an amino group, and the cytotoxic molecule is conjugated to the antibody through site-directed or undirected conjugation. The linker of the present disclosure may be selected from the following table:

Abbreviation	Full name	Feature
mc	Maleimidocaproyl	Non-cleavable [12]
mc-vc-pAB	Maleimidocaproyl valine citrulline p-amino-benzyl	Cleavable [12]
3-MPA	3-maleimido-propionic acid	Non-cleavable [12]
Mal-di-EG-OPFP	Perfluorophenyl 3-(2-(2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)ethoxy) ethoxy)propanoate	Non-cleavable [14]
Mal-di-EG-OSu	2,5-dioxopyrrolidin-1-yl 3-(2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy) propanoate	Non-cleavable [14]
Mal-Tri-EG-OSu	2,5-dioxopyrrolidin-1-yl 3-(2-(2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)ethoxy) propanoate	Non-cleavable [14]
Mal-Tetra-EG-OSu	2,5-dioxopyrrolidin-1-yl 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-oxo-7,10,13,16-tetraoxa-4 -azanonadecan-19-oate	Non-cleavable [14]
Br-di-EG-OSu	2,5-dioxopyrrolidin-1-yl 3-(2-(2-(2-bromoacetamido)ethoxy)ethoxy)propanoate	Non-cleavable [14]
Py-ds-prp-OSu	2,5-dioxopyrrolidin-1-yl 3-(pyridin-2-yldisulfanyl)propanoate	Reducible [14]
Py-ds-Prp-OPFP	Perfluorophenyl 3-(pyridin-2-yldisulfanyl)propanoate	Reducible [14]
Py-ds-dmBut-OSu	2,5-dioxopyrrolidin-1-yl 4-methyl-4-(pyridin-2-yldisulfanyl) pentanoate	Reducible [14]
Py-ds-dmBut-OPF	Perfluorophenyl 4-methyl-4-(pyridin-2-yldisulfanyl)pentanoate	Reducible [14]
SMCC	N-succinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate	Non-cleavable [13]
MBS	3-maleimidobenzoic acid N-hydroxysuccinimide ester	Non-cleavable [13]
SATA	S-(N-succinimidyl) thioacetate	Non-cleavable [13]
SPDP	N-succinimidyl 3-(2-pyridyldithio)propionate	Reducible [13]
SMPT	(N-succinimidyl carbonyl)-1-methyl-1-(2-pyridyldithio) toluene	Reducible [13]

[0021] The linker of the present disclosure is preferably Maleimido-Caproyl-Valine-Citrulline-p-Aminobenzyloxy (mc-vc-pAB) and Maleimidocaproyl (mc).

[0022] The linker of the present disclosure may also be triglycyl peptide linker, which is a new linker for ADCs developed in recent years (Rajeeva Singh et al., A New Triglycyl Peptide Linker for Antibody–Drug Conjugates (ADCs) with Improved Targeted Killing of Cancer Cells, MCT-16-002, Published June 2016). Alternatively, a glucuronide-tubulysin linker can be used (Patrick J. Burke et al., Glucuronide-linked antibody-tubulysin conjugates display activity in MDR^+ and heterogeneous tumor models, Molecular Cancer Therapeutics, 2018).

[0023] In another ~~aspect~~example, the antibody or the functional fragment thereof is derived from an antibody secreted by the hybridoma deposited at the China General Microbiological Culture Collection Center of China Committee for Culture Collection of Microorganisms, with an accession number CGMCC No. 8102 on August 22, 2013.

[0024] In another ~~aspect~~example, the antibody is a humanized antibody, preferably, the antibody is an antibody secreted by CHO cells deposited at the China Center for Type Culture Collection under the accession number CCTCC C2013170 on November 6, 2013.

[0025] In an ~~embodiment~~example, the antibody-drug conjugate used is named RC48-mc-vc-pAB-MMAE, which conforms to the structure of the general formula Ab-(L-U)n, in which RC48 (a humanized anti-HER2 monoclonal antibody) is coupled to MMAE through the linker mc-vc-pAB, and the number of coupling ranges from 1 to 8, including 1, 2, 3, 4, 5, 6, 7, 8 or a combination of antibody-drug conjugates with varying MMAE coupling numbers ranging from 1 to 8.

[0026] In the present disclosure, the urothelial carcinoma is locally advanced urothelial carcinoma that cannot be removed by surgery, locally advanced or metastatic urothelial carcinoma, HER2 (Human Epidermal Growth Factor Receptor 2, also called ErbB-2, C-erbB2, or HER2/neu) positive urothelial carcinoma, HER2 positive locally advanced or metastatic urothelial carcinoma.

CLAIMS

1. Use of an antibody-drug conjugate (ADC) in the preparation of a medicament for treating urothelial carcinoma, wherein the antibody-drug conjugate is an anti-HER2 antibody or a functional fragment thereof coupled to a cytotoxic molecule,

wherein the antibody-drug conjugate is represented by formula Ab-(L-U)n, wherein Ab is the antibody or the functional fragment thereof, L is a linker, U is a conjugated cytotoxic molecule, and n is an integer from 1 to 8, representing the number of therapeutic agent molecules bound to the antibody,

wherein the antibody or the functional fragment thereof is capable of competing with a defined antibody against the same epitope,

wherein the defined antibody comprises a heavy chain variable region and a light chain variable region defined by Complementarity Determining Region (CDR) sequences, and wherein

(i) the heavy chain variable region comprises three CDRs, wherein the amino acid sequences of the CDRs are respectively set forth in SEQ ID NO: 1, 2 and 3; and

(ii) the light chain variable region comprises three CDRs, wherein the amino acid sequences of the CDRs are respectively set forth in SEQ ID NO: 4, 5 and 6; and

wherein L is Maleimido-Caproyl-valine citrulline p-amino-benzyloxy (mc-vc-pAB), and

U is MMAE.

~~2. The use according to claim 1, wherein the cytotoxic molecule includes a tubulin inhibitor and a DNA damaging agent.~~

~~3. The use according to claim 2, wherein the tubulin inhibitor includes dolastatin and derivatives thereof, auristatin and derivatives thereof, maytansinoid and derivatives thereof; the DNA damaging agent includes calicheamicins, duocarmycins, tretracycline derivative PBD, and camptothecin derivative SN-38.~~

~~4. The use according to claim 1, the cytotoxic molecule includes amanitins, anthracyclines, baccatins, camptothecins, cemadotins, colchicines, colcimids, combretastatins, cryptophycins, dicodermolides, docetaxel, doxorubicin, echinomycins, eleutherobins, epothilones, estramustines, lexitropsins, maytansines, methotrexate, netropsins, puromycins, rhizoxins, taxanes, tubulysins, and vinca alkaloids.~~

~~5. The use according to claim 3, wherein the auristatin and derivatives thereof include MMAE, MMAF, and derivatives thereof; the maytansinoid and derivatives thereof include DM1, DM4, and the derivatives thereof.~~

~~6. The use according to any one of the preceding claims, wherein the antibody or the functional fragment thereof is capable of competing with a defined antibody against the same epitope,~~

~~wherein the defined antibody comprises a heavy chain variable region and a light chain variable region defined by CDR sequences, and wherein~~

~~(i) the heavy chain variable region comprises three CDRs, wherein the amino acid sequences of the CDRs are respectively set forth in SEQ ID NO: 1, 2 and 3; and~~

~~(ii) the light chain variable region comprises three CDRs, wherein the amino acid sequences of the CDRs are respectively set forth in SEQ ID NO: 4, 5 and 6.~~

~~7~~2. The use according to claim~~any one of claims~~ 1~~-5~~, wherein the antibody or the functional fragment thereof comprises a heavy chain variable region and a light chain variable region, wherein

(i) the heavy chain variable region comprises three CDRs, wherein the amino acid sequences of the CDRs are respectively set forth in SEQ ID NO: 1, 2 and 3; and

(ii) the light chain variable region comprises three CDRs, wherein the amino acid sequences of the CDRs are respectively set forth in SEQ ID NO: 4, 5 and 6.

~~8. The use according to any one of the preceding claims, wherein the antibody-drug conjugate is represented by formula Ab-(L-U)n, wherein Ab is the antibody or the functional fragment thereof, L is a linker, U is a conjugated cytotoxic molecule, and n is an integer from 1 to 8, representing the number of therapeutic agent molecules bound to the antibody.~~

~~9. The use according to claim 8, wherein the conjugated cytotoxic molecule includes DM1, DM3, DM4, MMAE, MMAF, AF (auristatin F) and derivatives thereof.~~

~~10. The use according to any one of the preceding claims, wherein the linker is attached to the antibody or the functional fragment thereof via a thiol and/or an amino.~~

~~11. The use according to claim 10, wherein the linker is selected from the group consisting of maleimidocaproyl valine citrulline p-amino-benzyl (mc-vc-pAB), maleimidocaproyl (mc), triglycyl peptide linker, 3-maleimido-propionic acid, Mal-di-EG-OPFP (perfluorophenyl 3-(2-(2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)ethoxy)ethoxy)propanoate), Mal-di-EG-OSu (2,5-dioxopyrrolidin-1-yl 3-(2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)propanoate), Mal-Tri-EG-OSu (2,5-dioxopyrrolidin-1-yl 3-(2-(2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)ethoxy)ethoxypropanoate), Mal-Tetra-EG-OSu (2,5-dioxopyrrolidin-1-yl 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) -3-oxo-7,10,13,16-tetraoxa-4-azanonadecan-19-oate), Br-di-EG-OSu (2,5-dioxopyrrolidin-1-yl 3-(2-(2-(2-bromoacetamido)ethoxy)ethoxy)propanoate), Py-ds-prp-OSu (2-5-dioxopyrrolidin-1-yl 3-(pyridine-2-yldisulfanyl)propanoate), Py-ds-Prp-OPEP (perfluorophenyl 3-(pyridine-2-yldisulfanyl)propanoate), Py-ds-dmBut-OSu (2,5-dioxopyrrolidin-1-yl 4-methyl-4-(pyridine-2-yldisulfanyl)pentanoate, Py-ds-dmBut-OPF (perfluorophenyl 4-methyl-4-(pyridine-2-yldisulfanyl)pentanoate), SMCC (N-succinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate), MBS (3-maleimidobenzoic acid N-hydroxysuccinimide ester), SATA (S-(N-succinimidyl)thioacetate), SPDP ((N-succinimidyl 3-(2-pyridyldithio)propionate), and SMPT ((N-~~

~~succinimidyloxy carbonyl)-1-methyl-1-(2-pyridyldithio)toluene).~~

~~12. The use according to any one of the preceding claims, wherein the cytotoxic molecule is conjugated to the antibody through site-directed or undirected conjugation.~~

~~13~~3. The use according to ~~any of the preceding~~ claim~~s~~ 1 or claim 2, wherein the antibody or the functional fragment thereof is murine, chimeric or humanized.

~~14~~4. The use according to any one of the preceding claims, wherein the antibody or the functional fragment thereof is derived from the antibody secreted by the hybridoma deposited at the China General Microbiological Culture Collection Center of China Committee for Culture Collection of Microorganisms on August 22, 2013, with an accession number of CGMCC No. 8102.

~~15~~5. The use according to any one of the preceding claims, wherein the antibody or the functional fragment thereof is derived from the~~is a humanized~~ antibody~~, preferably, the antibody is~~ secreted by the CHO cells deposited at the China Center for Type Culture Collection on November 6, 2013, with an accession number of CCTCC C2013170.

~~16~~6. The use according to any one of the preceding claims, the urothelial carcinoma is selected from the group consisting of locally developed urothelial carcinoma which cannot be removed by surgery, locally advanced or metastatic urothelial carcinoma, HER2-positive urothelial carcinoma and HER2-positive locally advanced or metastatic urothelial carcinoma.

~~17~~7. The use according to any one of the preceding claims, wherein the medicament further comprises a pharmaceutically acceptable carrier; the medicament is preferably a lyophilized formulation or a liquid formulation; the carrier is one or more selected from the group consisting of a stabilizer, a protective agent, a buffer, a lyoprotectant, an activity protective agent, a surfactant,

an adsorption carrier and an absorption promoter.

~~1~~8. The use according to any one of the preceding claims, the medicament can be administered intranasally, subcutaneously, intradermally, intramuscularly, or intravenously.

9. The use according to claim 6, wherein the urothelial carcinoma is a locally developed urothelial carcinoma which cannot be removed by surgery.

10. The use according to claim 6, wherein the urothelial carcinoma is a locally advanced or metastatic urothelial carcinoma.

11. The use according to claim 6, wherein the urothelial carcinoma is a HER2-positive urothelial carcinoma.

12. The use according to claim 6, wherein the urothelial carcinoma is a HER2-positive locally advanced or metastatic urothelial carcinoma.