

CYSTEINE MODIFIED ANTIBODY-DRUG CONJUGATE AND PREPARATION METHOD THEREOF CROSS REFERENCE TO RELATED APPLICATION

This application is a national stage application of International application number PCT/CN2017/104706, filed September 30, 2017, which claims the priority benefit of Chinese Patent Application No. 201610876568.9, filed on October 8, 2016, the entire disclosures of which are expressly incorporated by reference herein.

TECHNICAL FIELD

The present disclosure relates to therapeutic compounds and methods for the making thereof, and in particular to cysteine modified antibody-cytotoxin conjugates and methods of making thereof.

BACKGROUND

Antibody-drug conjugate (ADC) is a hotspot for targeted therapy. Two drugs, Adcetris and Kadcyra, have been approved for marketing in the United States, and have shown good clinical efficacy. There are more than 50 ADC drugs in Clinical trial stage.

SUMMARY

The present disclosure provides cysteine modified antibody-cytotoxin conjugates (TDC), the methods of making the antibody-cytotoxin conjugates, and methods of using thereof.

In one aspect, the application provides cysteine modified antibody-cytotoxin conjugates. In one embodiment, the cysteine insertion site includes one or more of the following three insertion sites or insertion positions in the target antibodies: light chain position 205 (Kabat numbering scheme, wherein the surrounding amino acid sequence comprises GLSSPCVTKSF, with C being the inserted cysteine), light chain 206 (Kabat numbering scheme, wherein the surrounding amino acid sequence comprises GLSSPVCTKSF, with C being the inserted cysteine), and heavy chain position 439 (474 Kabat numbering scheme, wherein the surrounding amino acid sequence comprises YTKSLSCLSPGK, with C being the inserted cysteine).

An antibody comprising one or more of the above cysteine insertion mutations retains the ability to bind the antigen as the parental antibody does (affinity). In one embodiment, the present disclosure provides the site-directed coupling of antibody-cytotoxin conjugate (TDC) by a cysteine thiol group with a linker-drug (i.e., linker-cytotoxin), wherein the thiol group is from the cysteine inserted into position 205 or/and position 206 of the light chain or/and position 439 of the heavy chain.

In one embodiment, the application provides cysteine modified antibody-cytotoxin conjugate, comprising an antibody that includes a site-specific inserted cysteine, where cysteine insertion site comprises one or more sites selected from the following three insertion sites: kappa/lambda light chain constant region position 205 (Kabat numbering scheme), kappa/lambda light chain constant region position 206 (Kabat numbering scheme), or the IgG antibody heavy chain constant region position 439 (474 Kabat numbering scheme).

CLAIMS

What is claimed is:

1. A cysteine modified antibody-cytotoxin conjugate, comprising an antibody and a cytotoxin, wherein the antibody comprises an inserted cysteine at a cysteine insertion site, wherein the cysteine insertion site is kappa/λ light chain constant region position 205 in Kabat numbering scheme, light chain constant region position 206 in Kabat numbering scheme, or IgG heavy chain constant region position 439 (474 in Kabat numbering scheme).
2. The cysteine modified antibody-cytotoxin conjugate of Claim 1, wherein the cysteine insertion site comprises an amino acid sequence selected from LC-205ins: GLSSPCVTKSF, LC-206ins: GLSSPVCTKSF, and HC-439ins: TQKSLSCLSPGK, and wherein the “C” is the inserted cysteine at the light chain constant region position 205, the light chain constant region position 206, or the heavy chain constant region position 439.
3. The cysteine modified antibody-cytotoxin conjugate of Claim 1, wherein the inserted cysteine comprises a free thiol group, wherein the cytotoxin is conjugated to the free thiol group through a linker, wherein the antibody comprises a light chain having an amino acid sequence selected from GLSSPCVTKSF and GLSSPVCTKSF, wherein the antibody comprises a heavy chain having an amino acid sequence of TQKSLSCLSPGK, and wherein the C is the inserted cysteine at the light chain position 205, the light chain position 206, or heavy chain position 439 of the antibody.
4. The cysteine modified antibody-cytotoxin conjugate of any one of Claims 1 to 3, wherein the antibody comprises a light chain, wherein the light chain comprises a kappa (κ) or lambda (λ) isotype.
5. The cysteine modified antibody-cytotoxin conjugate of any one of Claims 1 to 4, wherein the antibody comprises a heavy chain, wherein the heavy chain comprises IgG1, IgG2, IgG3 or IgG4.
6. The cysteine modified antibody-cytotoxin conjugate of Claim 1 or 2, wherein the inserted cysteine comprises a thiol group (-SH).
7. The cysteine modified antibody-cytotoxin conjugate of Claim 6, wherein the thiol group is configured for chemical conjugation.
8. The cysteine modified antibody-cytotoxin conjugate of any one of Claims 1 to 7, wherein the cytotoxin is selected from MMAE, MMAF, PBD, SN-38, Dox, or a derivative thereof.
9. A method for preparing the cysteine modified antibody-cytotoxin conjugate of any one of Claims 1 to 8, comprising:
 - reducing the antibody with a reducing agent to provide a reduced antibody, wherein the antibody comprises the inserted cysteine having its thiol group shielded with a shielding group,
 - removing the shielding group and the reducing agent through cation exchange

chromatography or ultrafiltration, wherein the reduced antibody having the shielding group removed comprises the inserted cysteine with a free thiol group,

- oxidizing the reduced antibody to provide an oxidized antibody having an inter-chain disulfide bond,
- conjugating a linker-cytotoxin with the free thiol group on the inserted cysteine to provide the cysteine modified antibody-cytotoxin conjugate, and
- removing unconjugated linker-cytotoxin by cation exchange chromatography or ultrafiltration.

10. Use of the cysteine modified antibody-cytotoxin conjugate according to any one of claims 1 to 8 in the preparation of a medicament for the treatment of cancer in a subject in need thereof.

11. The use of claim 10, wherein the cancer is squamous cell carcinoma, glioma, malignant glioma or pancreatic cancer.