Maleimido-Caproyl-Valine-Citrulline-p-AminoBenzyloxy (mc-VC-PAB), and the recombinant humanized Her2 monoclonal antibody-MMAE conjugate is formed by the monoclonal antibody and MMAE linked to the linker through cysteine. The drug has achieved good therapeutic effects in Her2 positive tumor.

[0010] The preliminary experiments showed that, after purification, the recombinant humanized anti-Her2 monoclonal antibody-MMAE conjugate has a poor solubility in the conventional buffer, and visible insoluble microparticles will appear, which does not meet the standards for injection. However, if a clinically effective therapeutic dose is to be achieved, the protein concentration needs to be above 5 mg/ml. Therefore, there is a need to solve the problem of insoluble particles while ensuring a certain effective concentration. In addition, when the protein solution is lyophilized under vacuum, it is necessary to add a lyoprotectant to protect the protein from being destroyed during lyophilization, and to ensure that the lyophilized powder has a good appearance at the same time. In order to avoid the slow degradation of lyophilized powder of the protein during long-term storage, it is necessary to add an appropriate protein stabilizer. Lots of experiments are needed to develop and determine the appropriate protein stabilizer.

[0011] Therefore, the purpose of the present invention is to develop a combination of adjuvants for anti-Her2 monoclonal antibody-MMAE conjugate through a wide range screening and concentration range screening to achieve the following technical effects: the anti-Her2 monoclonal antibody-MMAE conjugate can dissolve well before and after lyophilization, and both the insoluble microparticles and the visible precipitates meet the standards for human injection; in addition, the conjugate can be stable for a long time during lyophilization and storage, not be easily polymerized or degraded after reconstitution, and still maintains good biological activity.

SUMMARY

[0011a] In a first aspect, the present disclosure refers to an aqueous liquid pharmaceutical formulation of an antibody-drug conjugate, wherein

the formulation comprises the antibody-drug conjugate, a non-reducing sugar, an amino acid, and a solubilizer,

the non-reducing sugar is selected from mannitol, sucrose, trehalose, and a combination thereof,

the amino acid is selected from histidine, alanine, arginine, glycine, glutamic acid, a hydrochloride of the above amino acids, and a combination thereof, and

the solubilizer is selected from glycerol, Tween 80, and a combination thereof.

[0011b] In another aspect, the present disclosure refers to a lyophilized pharmaceutical formulation of an antibody-drug conjugate, wherein the lyophilized pharmaceutical formulation is obtained by lyophilization of the aqueous liquid pharmaceutical formulation disclosed herein.

[0011c] In yet another aspect, the present disclosure refers to a use of the pharmaceutical formulation disclosed herein in the manufacture of a medicament for treating a disease caused by abnormal expression of Her2, preferably cancer, further preferably Her2 positive cancer, and most preferably breast cancer, ovarian cancer, gastric cancer, urothelial cancer, gastroesophageal cancer, oesophageal cancer, endometrial cancer, lung cancer, or bladder cancer.

[0011d] In a further aspect, the present disclosure refers to a method for preparing a pharmaceutical formulation of an antibody-drug conjugate, comprising:

(1) preparing the formulation disclosed herein; and

(2) evaluating the stability of the antibody-drug conjugate in the formulation.

[0012] The present disclosure provides an aqueous liquid pharmaceutical formulation of an antibody-drug conjugate, wherein the formulation comprises the antibody-drug conjugate, a non-reducing sugar, an amino acid, and a solubilizer; wherein the non-reducing sugar is selected from mannitol, sucrose, trehalose, and a combination thereof; the amino acid is selected from

histidine, alanine, arginine, glycine, glutamic acid, and a combination thereof; and the solubilizer is selected from glycerol, Tween 80, and a combination thereof.

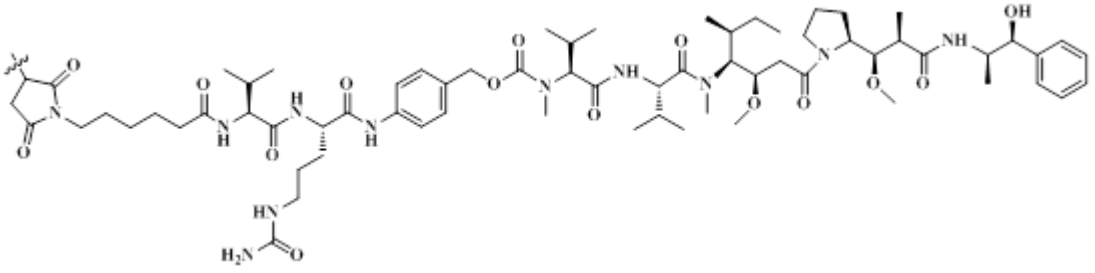
[0013] In some examples, the concentration of the mannitol is 100-300 mmol/L, preferably 190-300 mmol/L, more preferably 200-260 mmol/L, most preferably 240-260 mmol/L. The concentration of the sucrose is 0-100 mmol/L, preferably 40-100 mmol/L, more preferably 60-100 mmol/L, most preferably 40-60 mmol/L.

[0014] In some examples, the histidine is histidine hydrochloride at a concentration of 0-100 mmol/L, preferably 5-50 mmol/L, more preferably 5-20 mmol/L, most preferably 10 mmol/L. The arginine is arginine hydrochloride at a concentration of 0-160 mmol/L, preferably 20-100 mmol/L, more preferably 30-90 mmol/L, most preferably about 35 mmol/L.

[0015] In some examples, the content of the glycerol is 0-1%, preferably 0.2-0.5% (w/v). The mass percentage of Tween 80 is 0-0.02% (w/v).

[0016] Further, the antibody in the antibody-drug conjugate is an anti-HER2 monoclonal antibody; and the drug to which the antibody is conjugated is MMAE, MMAF, DM1, DM4, or a derivative thereof.

[0017] Further, the anti-HER2 antibody-drug conjugate is an anti-HER2 monoclonal antibody-vc-MMAE, wherein the anti-HER2 monoclonal antibody is connected to MMAE via a linker vc, and the structure formed by the connection of the linker and MMAE is:



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[0018] Further, the anti-HER2 monoclonal antibody comprises a heavy chain and a light chain,

(i) the heavy chain comprises CDRs 1-3 having amino acid sequences shown in SEQ ID NO: 1, 2 and 3, respectively; and/or

(ii) the light chain comprises CDRs 1-3 having amino acid sequences shown in SEQ ID NO: 4, 5 and 6, respectively.

[0019] Further, the monoclonal antibody is preferably a chimeric antibody or a humanized antibody.

[0020] In some examples, the concentration of the anti-HER2 monoclonal antibody-vc-MMAE is 5-30 mg/ml.

[0021] In some examples, the non-reducing sugar is 240-260 mmol/L of mannitol and/or 40 mmol/L-60 mmol/L of sucrose, the amino acid is 8-12 mmol/L of histidine hydrochloride, and the solubilizer is 0-0.02% (w/v) of Tween 80.

[0022] In some examples, wherein the non-reducing sugar is about 260 mmol/L of mannitol and about 40 mmol/L of sucrose, the amino acid is about 10 mmol/L of histidine hydrochloride, the concentration of the antibody-drug conjugate is about 10 mg/ml, and the solubilizer is about 0.02% (w/v) of Tween 80.

[0023] In some examples, wherein the non-reducing sugar is about 240 mmol/L of mannitol and about 60 mmol/L of sucrose, the amino acid is about 10 mmol/L of histidine hydrochloride, the concentration of the antibody-drug conjugate is about 10 mg/ml, and the solubilizer is about 0.02% (w/v) of Tween 80.

[0024] Further, the formulation has a pH of 4.5-7, preferably 5.6-6.8, more preferably 5.6-6.5, 5.6-6.4, 5.6-6.3, 6.1-6.4, or 6.1-6.3.

[0025] The pH of the formulation is adjusted with NaOH or hydrochloric acid.

[0026] In some examples, the lyophilized pharmaceutical formulation is obtained by lyophilization of the above aqueous liquid pharmaceutical formulation.

[0027] Further, before lyophilization, the aqueous liquid pharmaceutical formulation comprises about 260 mmol/L of mannitol, about 40 mmol/L of sucrose, about 10 mmol/L of histidine hydrochloride, about 0.02% (w/v) of Tween 80, and about 10 mg/ml of anti-HER2 monoclonal antibody-vc-MMAE, and has a pH of 5.6-6.8.

[0028] Further, before lyophilization, the aqueous liquid pharmaceutical formulation comprises about 240 mmol/L of mannitol, about 60 mmol/L of sucrose, about 10 mmol/L of histidine hydrochloride, 0.02% of Tween 80, and about 10 mg/ml of anti-HER2 monoclonal antibody-vc-MMAE, and has a pH of 5.6-6.8.

[0029] Further, before lyophilization, the non-reducing sugars comprised in the aqueous liquid pharmaceutical formulation are mannitol and sucrose at concentrations of about 47.36 mg/ml and about 13.69 mg/ml, respectively, and the amino acid is histidine hydrochloride at a concentration of about 2.10 mg/ml, and the solubilizer is Tween 80 at a content of about 0.02% (w/v).

[0030] Further, before lyophilization, the non-reducing sugars comprised in the aqueous liquid pharmaceutical formulation are mannitol and sucrose at concentrations of about 43.72 mg/ml and about 20.54 mg/ml, respectively, and the amino acid is histidine hydrochloride at a concentration of about 2.10 mg/ml, and the solubilizer is Tween 80 at a content of about 0.02% (w/v).

[0031] The present disclosure further provides the use of the above pharmaceutical formulations in the manufacture of a medicament for treating a disease caused by abnormal expression of Her2, preferably cancer; further preferably Her2 positive cancer; further preferably breast cancer, ovarian cancer, gastric cancer, urothelial cancer, gastroesophageal cancer, oesophageal cancer, endometrial cancer, lung cancer, or bladder cancer (Reference 3: Human Epidermal Growth Factor Receptor 2 (HER2) in Cancers: Overexpression and Therapeutic Implications, Nida Iqbal and Naveed Iqbal, Molecular Biology International, Volume 2014, Article ID 852748; and Reference 4: CN201810998055.4).

[0032] The present disclosure further provides a method for preparing a pharmaceutical formulation for an antibody-drug conjugate, comprising:

(1) preparing the formulation of any one of the above; and

(2) evaluating the stability of the antibody-drug conjugate in the formulation.

[0033] BRIEF DESCRIPTION OF DRAWINGS

[0034] Figure 1 shows SDS-PAGE electrophoresis of the naked antibody after purification, in which, 1-2: non-reducing electrophoresis; and 3-4: reducing electrophoresis. The result in Figure 1 shows that the purity of the monoclonal antibody obtained in the experiment meets the requirements of further experiments.

[0035] Figure 2 shows 10% SDS-PAGE electrophoresis of ADC after conjugation, in which, Na: non-reducing electrophoresis; Nb: non-reducing electrophoresis after the antibody is reduced