

PHARMACEUTICAL COMPOSITION

FIELD OF THE INVENTION

[0001] The present invention relates to novel Antibody Drug Conjugate (ADC) formulations. In particular, the invention relates to pharmaceutical compositions of a particular ADC, to methods of manufacturing the compositions, to kits including the compositions, to containers including the compositions, and to the use of said pharmaceutical compositions as a medicament and for the treatment of diseases, such as cancer.

BACKGROUND OF THE INVENTION

[0002] The present application is directed to particular pharmaceutical compositions comprising an antibody drug conjugate targeting trophoblast cell surface antigen 2 (TROP2).

[0003] TROP2 is a cell surface glycoprotein and is highly expressed in a variety of tumours including breast, lung, pancreatic, ovarian, and prostate cancer. The transmembrane protein plays a role in tumour cell proliferation and is a validated oncology target.

[0004] A number of TROP2 targeting antibody drug conjugates are known and have shown clinical activity in patients with TROP2-expressing tumours. DS-1062 is an ADC originally developed by Daiichi Sankyo using its DXd technology. Trodelvy® (sacituzumab govitecan-hziy) was the first FDA-approved anti-TROP2 ADC and is indicated for the treatment of adult patients with unresectable locally advanced or metastatic triple-negative breast cancer (TNBC) who have received two or more prior systemic therapies, at least one of them for metastatic disease. Trodelvy® is also approved in the U.S. for the treatment of adult patients with locally advanced or metastatic urothelial cancer (UC) who have previously received a platinum-containing chemotherapy and either programmed death receptor-1 (PD-1) or programmed death-ligand 1 (PD-L1) inhibitor. Trodelvy® consists of a fully humanized hRS7 IgG1κ antibody targeted against TROP2 conjugated to SN-38, the active metabolite of irinotecan.

[0005] Further TROP2 targeted therapies are needed and the applicant has developed a novel TROP2 directed ADC with improved properties. The ADC has been disclosed in PCT International Application No. PCT/CN2023/107444, the entire contents of which are incorporated herein by reference. The ADC utilises a stable linker with a next generation camptothecin analogue and is prepared using a ligase-dependent conjugation technology platform. The ADC has been tested and pre-clinical data indicates that the ADC has superior linker stability and *in vitro* and *in vivo* efficacy, and an excellent safety profile, when compared to existing ADCs, such as DS-1062 and Trodelvy®.

[0006] Whilst appreciable technological advances have culminated in the discovery of new ADCs, development of these conjugates into suitable and acceptable dosage forms for therapeutic purposes remains challenging and formulation development is highly complex.

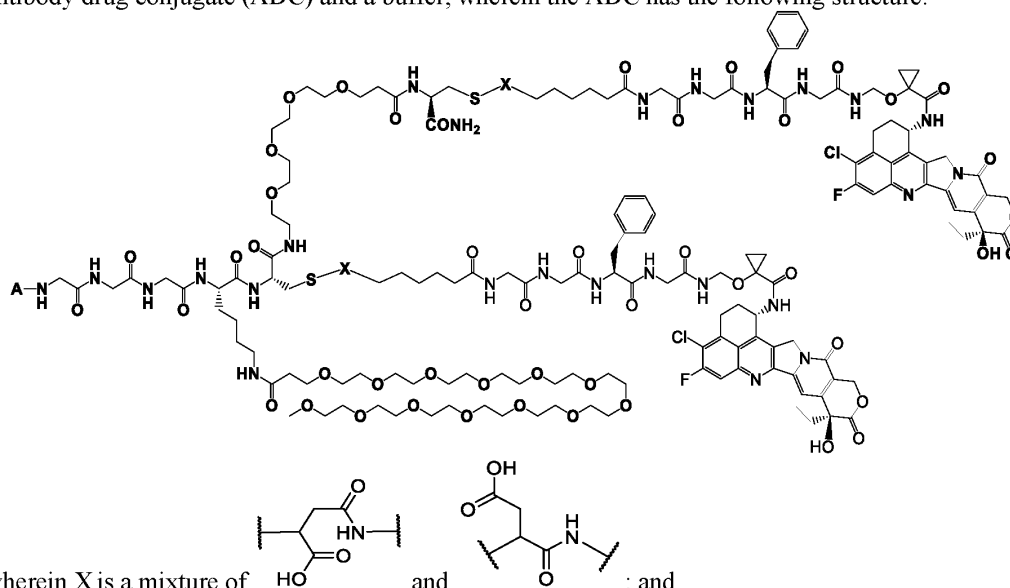
[0007] Antibodies are prone to degradation such as oxidation, deamidation and fragmentation as well as particle formation and aggregation. In addition, after drug conjugation, the properties of antibodies are often altered, leading to additional challenges. ADCs are complex engineered therapeutics and have more heterogenous structures than unconjugated antibodies. This complexity causes makes it difficult to develop viable pharmaceutical compositions and many formulations of a given ADC, especially aqueous based compositions, are unstable over prolonged periods and/or under stressed conditions owing to the large variety of possible degradation pathways open to such compositions. Degradation may involve aggregation of the antibody conjugate molecules, precipitation, adsorption of molecules at the interface of water and air or at the contact surface of any packaging material, inadequate control and regulation of osmotic pressure, oxidation, photo-oxidation, hydrolysis, inadequate stabilisation and maintenance of pH, protein fragmentation and/or protein unfolding. The chemical cytotoxic payloads and linkers used in ADCs present different physicochemical challenges compared to antibodies thus leading to further complexities, such as payload stability and shedding, for formulation development.

[0008] Any, some, or all of the above factors can lead to either an unviable drug product, which may be unsafe for use, or a drug product with variable and unpredictable performance, especially in view of the stresses batches of drug product may be exposed to during manufacture, transport, and storage.

[0009] There is an ongoing need for novel and improved ADC containing pharmaceutical compositions for treating diseases, such as cancer, and the present invention was devised with the foregoing in mind.

SUMMARY OF THE INVENTION

[0010] In a first aspect, the present invention provides a pharmaceutical composition comprising an antibody drug conjugate (ADC) and a buffer, wherein the ADC has the following structure:



wherein the linker and antibody or antibody fragment are connected by an amide bond to at least one light chain of the antibody or antibody fragment;

and wherein the pharmaceutical composition is a liquid and has a pH of 5.4 to 6.5.

[0011] The ADC shown above is an anti-TROP2 ADC, consisting of an antibody targeting TROP2 (GQhRS7) or a fragment thereof, wherein at least one light chain of the antibody or a fragment thereof is conjugated via a linker to two cytotoxic payload molecules. Conveniently, the linker and antibody or fragment thereof are connected by an amide bond at the C-terminus of the at least one light chain of the antibody or fragment thereof.

[0012] In an embodiment, A is the anti-TROP2 antibody and each light chain of the antibody is connected by an amide bond at the C-terminus of the light chain to a linker connected to two cytotoxic payload molecules. It is to be understood that in this embodiment, the ADC therefore consists of an antibody targeting TROP2 (GQhRS7), wherein a first light chain of the antibody is conjugated via a linker to two cytotoxic payload molecules and a second light chain of the antibody is also conjugated via a separate but identical linker to a further two cytotoxic payload molecules.

[0013] The pharmaceutical composition according to the first aspect of the invention has been found to offer certain advantages important to the delivery of a viable ADC formulation. By control of the pH of the composition, when formulated as a liquid, it has been found that it is possible to minimise payload shedding from the ADC and ADC aggregation, and maintain a more stable charge heterogeneity of the composition.

[0014] In a second aspect of the invention, there is provided a process for manufacturing a pharmaceutical composition according to the first aspect.

[0015] In a third aspect of the invention, there is provided a lyophilized formulation comprising the ADC and a buffer, wherein the formulation can be reconstituted to form the pharmaceutical composition according to the first aspect.

[0016] In a fourth aspect of the invention, there is provided a process for manufacturing a lyophilized according to the third aspect.

[0017] In a fifth aspect of the invention, there is provided a container comprising a pharmaceutical composition according to the first aspect, or a lyophilized formulation according to the third aspect, wherein the container can be a vial, single-use vial, light-protected vial, ampoule, syringe, pre-filled syringe, injection pen or intravenous infusion bag.

[0018] In a sixth aspect of the invention, there is provided a pharmaceutical composition according to the first aspect, or a lyophilized formulation according to the third aspect, for use as a medicament.

[0019] In a seventh aspect of the invention, there is provided a method of treating cancer or an autoimmune disease in a subject in need thereof, the method comprises administering to the subject a therapeutically effective amount of a pharmaceutical composition according to the first aspect, or a lyophilized formulation according to the third aspect. In a suitable embodiment the cancer is a TROP2-associated tumour. In a suitable embodiment the cancer is selected from fibrosarcoma, myosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendothelial sarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon cancer, pancreatic cancer, breast cancer, thyroid cancer, endometrial cancer, melanoma, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous carcinoma, papillary carcinoma, papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, bronchial carcinoma, renal cell carcinoma, liver cancer, bile duct cancer, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung cancer, small cell lung cancer, bladder cancer, epithelial cancer, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma and retinoblastoma.

[0020] In an eighth aspect of the invention, there is provided a kit of parts comprising a pharmaceutical composition according to the first aspect, or a lyophilized formulation according to the third aspect, in a container and, optionally, a set of instructions with directions regarding the administration of the pharmaceutical composition, or the lyophilized formulation.

[0021] Preferred, suitable, and optional features of any one particular aspect of the present invention are also preferred, suitable, and optional features of any other aspect.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] Figure 1 shows the Trop2 binding affinity curves for ADC, the comparative ADC Trodelvy® and antibody GQhRS7.

[0023] Figure 2 shows the effect of ADC, comparative ADC DS-1062a and antibody GQhRS7 at various concentrations on the viability of human pancreatic cancer cells BxPC-3 as a percentage of control.

[0024] Figure 3 shows the effect of ADC, comparative ADC DS-1062a and antibody GQhRS7 at various concentrations on the viability of pharyngeal squamous carcinoma cells FaDu as a percentage of control.

[0025] Figure 4 shows the effect of ADC, comparative ADC DS-1062a and antibody GQhRS7 at various concentrations on the viability of gastric cancer cells NCI-N87 as a percentage of control.

[0026] Figure 5 shows the inhibitory effect on tumour volume of ADC at 0.5, 1.5, and 4.5 mg/kg, the comparative ADCs Trodelvy® and DS-1062a at 4.5 mg/kg, and vehicle when dosed intravenously to mice bearing MDA-MB-468 Trop-2 positive human breast cancer xenograft tumours.

DETAILED DESCRIPTION OF THE INVENTION

[0027] The specific embodiments are provided below to illustrate technical contents of the present disclosure. Those skilled in the art can easily understand other advantages and effects of the present disclosure through the contents disclosed in the specification. The present disclosure can also be implemented or applied through other different specific embodiments. Various modifications and variations can be made by those skilled in the art without departing from the spirit of the present disclosure.

Definitions

[0028] Unless otherwise defined hereinafter, all technical and scientific terms used herein have the same meaning as commonly understood by those skilled in the art. The techniques used herein refer to those that are generally understood in the art, including the variants and equivalent substitutions that are obvious to those skilled in the art. While the following terms are believed to be readily comprehensible by those skilled in the art, the following definitions are set forth to better illustrate the present disclosure. When a trade name is present herein, it refers to the corresponding commodity or the active ingredient

thereof. All patents, published patent applications and publications cited herein are hereby incorporated by reference.

[0029] As used herein, the term “antibody” includes the intact antibody and fragments of the antibody as long as they have the desired biological activity, e.g., ability to bind TROP2. Suitably, the antibody is GQhRS7, which is an anti-TROP2 antibody comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein:

the V_H comprises:

- (i) HCDR1 comprising the amino acid sequence NYGMN (SEQ ID NO: 4);
 - (ii) HCDR2 comprising the amino acid sequence WINTYTGEPTYTDDFKG (SEQ ID NO: 5);
 - (iii) HCDR3 comprising the amino acid sequence GGFSSYWYFDV (SEQ ID NO: 6);
- and

the V_L comprises:

- (i) LCDR1 comprising the amino acid sequence KASQDVSIABA (SEQ ID No: 7);
- (ii) LCDR2 comprising the amino acid sequence SASRYT (SEQ ID NO: 8);
- (iii) LCDR3 comprising the amino acid sequence of QQHYITPLT (SEQ ID NO: 9);

wherein the V_L region also comprises a recognition sequence of a ligase donor substrate. Suitably, the recognition sequence of a ligase donor substrate is attached to the C-terminus of the V_L region.

Suitably, the recognition sequence of a ligase donor substrate comprises the sequence LPETGG (SEQ ID NO: 10). Suitably, the recognition sequence of a ligase donor substrate is attached to the V_L region via a spacer. Suitably, the linker has the sequence GA.

[0030] In a particular embodiment, the antibody GQhRS7 comprises a heavy chain sequence (SEQ ID NO: 1) and a light chain sequence (SEQ ID NO: 2) as follows:

QVQLQQSGSELKKPGASVKVSKASGYTFTNYGMNWVKQAPGQGLKWMGWINTYTGEPT
YTDDFKGRFAFLDTSVSTAYLQISSLKADDTAVYFCARGGFGSSYWYFDVWGQGLSLTVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVPEPKSCDKTHTCPPCPAPELGGPSVFLFP
PKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL
LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMH
EALHNHYTQKSLSLSPGK (SEQ ID NO: 1)

DIQLTQSPSSLSASVGDRVSITCKASQDVSIABAWYQQKPGKAPKLLIYSASYRTGVPDRFS
GSGSGTDFLTITSLQPEDFAVYYCQQHYITPLTFGAGTKVEIKRTVAAPSVFIFPPSDEQLKSG
TASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSSTLTLSKADYEKHKH
VYACEVTHQGLSSPVTKSFNRGECGALPETGG (SEQ ID NO: 2)

[0031] The term “buffer” means a component that resists changes in pH when acid or alkali is added to it. A person of skill in the art will be able to choose a suitable buffer to achieve the desired pH range. A buffer typically consists of a weak acid and a salt thereof, or a weak alkali and a salt thereof. Non-limiting examples of buffers include acetate buffer (acetic acid and sodium acetate), citrate buffer (citric acid and sodium citrate), histidine buffer (histidine and histidine hydrochloride), succinate buffer (succinic acid and sodium succinate), phosphate buffer (potassium phosphate monobasic and sodium phosphate dibasic) and bis-tris methane buffer (BTM; zwitterionic buffer).

[0032] The term “drug antibody ratio” or “DAR” means the average number of drug molecules conjugated to an antibody. Suitable methods for determining the DAR of the ADC may be determined by a person of skill in the art, and include UV/Vis spectroscopy, hydrophobic interaction chromatography (HIC), reversed phase HPLC (RP-HPLC) and mass spectrometry.

[0033] As used herein, “patient” or “subject” refers to a mammal, including domestic pets, animals kept as livestock and zoo animals. Conveniently, the mammal is an ape, such as a human. Most conveniently, the “patient” or “subject” is a human.

[0034] It is to be appreciated that references to “treating” or “treatment” include alleviation of established symptoms of a condition. “Treating” or “treatment” of a state, disorder or condition therefore includes: (1) delaying the appearance of clinical symptoms of the state, disorder or condition developing in a human that may be afflicted with, disorder or condition but does not yet experience or display clinical or subclinical symptoms of the state, disorder or condition, (2) inhibiting the state, disorder or condition, i.e., arresting, reducing or delaying the development of the disease or a relapse thereof (in case of maintenance treatment) or at least one clinical or subclinical symptom thereof, or (3) relieving

or attenuating the disease, i.e., causing regression of the state, disorder or condition or at least one of its clinical or subclinical symptoms.

[0035] A “therapeutically effective amount” means the amount of a compound that, when administered to a mammal for treating a disease, is sufficient to effect such treatment for the disease. The “therapeutically effective amount” will vary depending on the compound, the disease and its severity and the age, weight, etc., of the mammal to be treated.

[0036] As used herein, where the term “about” is before a quantitative value, the present teachings also include the specific quantitative value itself, unless specifically stated otherwise. Further, the term “about” refers to a $\pm 10\%$ variation from the nominal value unless otherwise indicated or inferred.

[0037] At various places in the present specification, values are disclosed in groups or in ranges. It is specifically intended that the description include all individual sub-combination of the members of such groups and ranges and any combination of the various endpoints of such groups or ranges. For example, an integer in the range of 0 to 40 is specifically intended to individually disclose 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, and 40, and an integer in the range of 1 to 20 is specifically intended to individually disclose 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20.

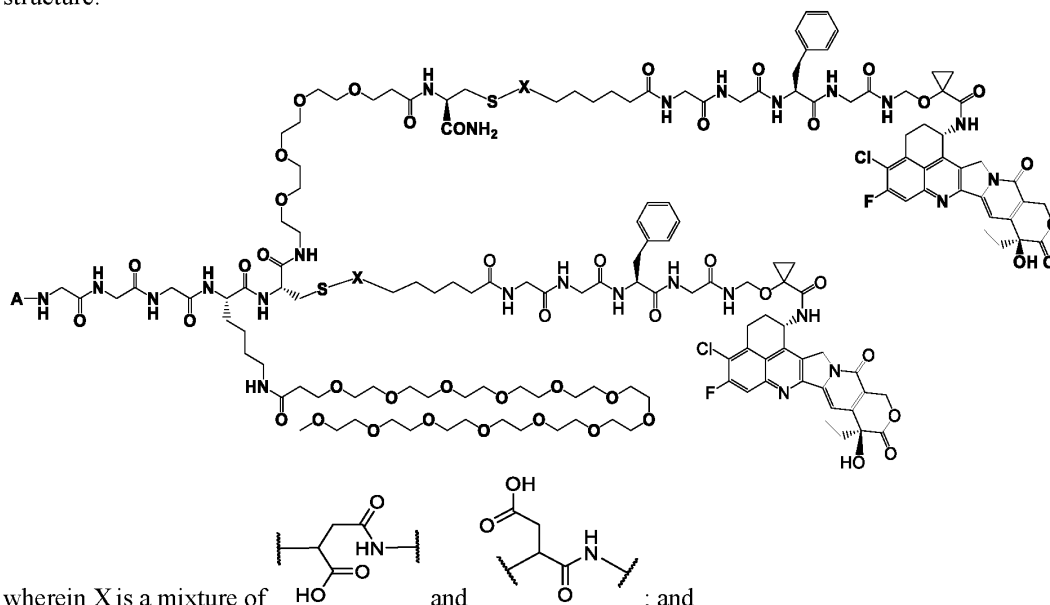
[0038] As used herein, where compositions are described as having, including, or comprising specific components, or where processes are described as having, including, or comprising specific process steps, it is contemplated that compositions of the present teachings also consist essentially of, or consist of, the recited components, and that the processes of the present teachings also consist essentially of, or consist of, the recited process steps.

[0039] The term “optional” or “optionally” means the event described subsequent thereto may, but not necessarily happen, and the description includes the cases wherein said event or circumstance happens or does not happen.

[0040] The use of any and all examples, or exemplary language herein, for example, “such as,” “including,” or “for example,” is intended merely to illustrate better the present teachings and does not pose a limitation on the scope of the invention unless claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the present teachings.

Pharmaceutical Compositions

[0041] According to a first aspect of the invention, there is provided a pharmaceutical composition comprising an antibody drug conjugate (ADC) and a buffer, wherein the ADC has the following structure:



wherein the linker and antibody or antibody fragment are connected by an amide bond to at least one light chain of the antibody or antibody fragment;

and wherein the pharmaceutical composition is a liquid and has a pH of 5.4 to 6.5.

[0042] In accordance with an embodiment of the first aspect of the invention, the linker and GQhRS7 antibody are connected by an amide bond at the C-terminus of at least one, conveniently each, light chain of the antibody. The ADC is prepared by the ligase-catalysed site-specific conjugation of the GQhRS7 antibody and the linker-payload compound. The (Glycine)₃ moiety of the linker attached to the antibody is a recognition sequence of a ligase acceptor substrate, which facilitates enzyme-catalysed coupling of the linker-payload with the GQhRS7 antibody under the catalysis of the ligase. In order to connect with the (Glycine)₃ moiety of the linker, the GQhRS7 antibody comprises a terminal modification at the C-terminus of each light chain comprising a spacer and the corresponding recognition sequence of a ligase donor substrate. Specifically, the sequence of GQhRS7 is based on the amino acid sequence of hRS7 (Sacituzumab), and GALPETGG (SEQ ID NO: 11) has been introduced at the C-terminus of each light chain, wherein LPETGG is the recognition sequence of the ligase donor substrate, and GA is a spacer sequence. It should be understood that, when the GQhRS7 antibody conjugates with the (Glycine)₃ moiety of the linker-payload under the catalysis of the ligase, the recognition sequence of the ligase acceptor substrate and the recognition sequence of the ligase donor substrate react with each other and form a resulting sequence. When the GQhRS7 antibody conjugates with (Glycine)₃, which is the corresponding recognition sequence of the ligase acceptor substrate, the upstream peptide bond of GG in the LPETGG sequence is cleaved by Sortase A, and the resulting intermediate is linked to the free N-terminal of G₃ to generate a new peptide bond. The resulting sequence is LPETG₃ (SEQ ID NO: 12). Suitably, when the GQhRS7 antibody has been conjugated with the (Glycine)₃ moiety of the linker, the light chain amino acid sequence SEQ ID NO: 2 is modified such that the terminal GG amino acids have been omitted to provide a light chain sequence (SEQ ID NO: 3) as follows:

DIQLTQSPSSLSASVGDRVSITCKASQDVSI AVAWYQQKPGKAPKLLIYSASYRYTGVPDRFS
GSGSGTDFTLTISLQPEDFAVYYCQQHYITPLTFGAGTKVEIKRTVAAPSVFIFPPSDEQLKSG
TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGECGALPET (SEQ ID NO: 3)

[0043] Thus, suitably the antibody moiety “A” in claim 1 comprises SEQ ID NO: 1 and SEQ ID NO: 3.

[0044] The ADC typically has an average drug to antibody ratio (DAR) in the range of 2 to 4, or conveniently about 3.5. The pharmaceutical composition according to the present invention has been found to provide a formulation with good stability in terms of maintaining the DAR of the ADC. Therefore, in an embodiment there is provided a pharmaceutical composition according to the first aspect, wherein the ADC has an average drug to antibody ratio (DAR) in the range of 2 to 4, such as 2.5 to 4. In an embodiment, the ADC has an average drug to antibody ratio (DAR) in the range of 3 to 4, such as 3.1 to 3.9, 3.2 to 3.8, 3.3 to 3.7, or 3.4 to 3.6. In an embodiment, the ADC has an average drug to antibody ratio (DAR) of about 3.5.

[0045] In an embodiment there is provided a pharmaceutical composition according to the first aspect, wherein after the composition has been stored at 25 °C for at least 4 weeks the ADC has an average drug to antibody ratio (DAR) which is the same (± 0.2) as the DAR at T=0 (start of the sample storage). In an embodiment, the DAR of the ADC after at least 3 months storage at 25 °C is the same (± 0.1) as the DAR at T=0. In an embodiment there is provided a pharmaceutical composition according to the first aspect, wherein after the composition has been stored at 40 °C for at least 2 weeks the ADC has an average drug to antibody ratio (DAR) which is the same (± 0.2) as the DAR at T=0 (start of the sample storage). In an embodiment, the DAR of the ADC after at least 4 weeks storage at 40 °C is the same (± 0.1) as the DAR at T=0.

[0046] In an embodiment there is provided a pharmaceutical composition according to the first aspect, wherein after the composition has been stored at 25 °C for at least 4 weeks the ADC has an average drug to antibody ratio (DAR) in the range of 2 to 4, and which DAR is the same (± 0.2) as the DAR at T=0 (start of the sample storage). In an embodiment there is provided a pharmaceutical composition according to the first aspect, wherein after the composition has been stored at 40 °C for at least 4 weeks the ADC has an average drug to antibody ratio (DAR) in the range of 3 to 4, and which DAR is the same (± 0.1) as the DAR at T=0.

[0047] In an embodiment, the pharmaceutical composition is an aqueous liquid composition. In an embodiment, the buffer is present as an aqueous buffer solution. In an embodiment, the buffer is an aqueous buffer solution with a pH in the range 5.4 to 6.5.

[0048] In an embodiment, there is provided a pharmaceutical composition according to the first aspect, wherein the ADC is dissolved in the pharmaceutical composition at a concentration of about 1 to 100 mg/mL. It is to be understood that the concentration refers to the concentration of the protein of the ADC dissolved in the pharmaceutical composition. Conveniently, the ADC is dissolved in the pharmaceutical composition at a concentration of about 5 to 100 mg/mL. Conveniently, the ADC is dissolved in the pharmaceutical composition at a concentration of about 5 to 50 mg/mL, 10 to 40 mg/mL, 10 to 30 mg/mL; more conveniently at about 15 to 25 mg/mL, or 18 to 22 mg/mL. In an embodiment, the ADC is dissolved in the pharmaceutical composition at a concentration of about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, or about 30 mg/mL. Most conveniently, the ADC is dissolved in the pharmaceutical composition at a concentration of about 20 mg/mL.

[0049] It has been found that by control of the pH of the pharmaceutical composition when formulated as a liquid, it is possible to minimise payload shedding from the ADC and also minimise ADC aggregation. In particular it has been discovered that when the pH is too low (pH < about 5.4) increased ADC aggregation tends to be observed, and when the pH is too high (pH > about 6.5) increased payload shedding from the conjugate tends to occur. In an embodiment the pharmaceutical composition is a liquid and has a pH of 5.4 to 6.5. In an embodiment, the pH of the composition is about 5.5 to 6.4, about 5.6 to 6.4, about 5.7 to 6.4, about 5.8 to 6.4, about 5.9 to 6.4, about 6.0 to 6.4, about 5.5 to 6.3, about 5.5 to 6.2, about 5.5 to 6.1, about 5.6 to 6.3, about 5.6 to 6.2, about 5.6 to 6.1, about 5.7 to 6.3, about 5.7 to 6.2, about 5.7 to 6.1, about 5.8 to 6.3, about 5.8 to 6.2, about 5.8 to 6.1, about 5.5 to 6.5, about 5.6 to 6.5, about 5.7 to 6.5, about 5.8 to 6.5, about 5.9 to 6.5, or about 6.0 to 6.5. Conveniently, the pH of the composition is about 5.5 to 6.2, or more conveniently about 5.8 to 6.2. Conveniently, the pH of the composition is about 5.9 to 6.3.

[0050] In an embodiment, the pH of the composition is 5.4, about 5.5, about 5.6, about 5.7, about 5.8, about 5.9, about 6.0, about 6.1, about 6.2, about 6.3, about 6.4, or 6.5.

[0051] Furthermore it has been discovered that, in terms of the charge heterogeneity of the composition, acidic components and basic components increase more rapidly at lower pH. Conveniently, the pH of the composition is about 6.0 to 6.2, or about 6.1. Conveniently, the pH of the composition is 6.1.

[0052] A skilled person will appreciate that the buffer present in the pharmaceutical composition according to the first aspect may be any buffer, or buffer system, suitable for providing a pH of about 5.4 to 6.5. Suitable buffers may include citrate buffer, succinate buffer, histidine buffer, phosphate buffer, acetate buffer and malate buffer. In an embodiment, the buffer is selected from citrate buffer, succinate buffer, histidine buffer, and acetate buffer.

[0053] It has been discovered that a histidine buffer system may offer certain advantages over other buffer systems. The citrate and succinate buffers are more likely to exhibit increased ADC aggregation on storage; certain compositions containing the citrate buffers gave visible particulate matter under accelerated storage conditions; and certain citrate and succinate buffer compositions gave higher insoluble particle levels. Therefore, in a convenient embodiment, the buffer is a histidine buffer. In a more convenient embodiment, the buffer is a histidine buffer, wherein the histidine buffer comprises a mixture of L-histidine and L-histidine monohydrochloride. In an embodiment, the pharmaceutical composition according to the first aspect is substantially free from citrate or succinate buffers. In an embodiment, the pharmaceutical composition according to the first aspect is free from citrate or succinate buffers.

[0054] In an embodiment, the pharmaceutical composition according to the first aspect comprises buffer components. In this context buffer components refers to two or more components that provide the pH buffering capability of the buffer. In an embodiment, the buffer components are a weak acid (such as citric acid, acetic acid, succinic acid or malic acid) and a conjugate salt thereof. In an embodiment, the buffer components are a weak alkali (such as histidine) and a conjugate salt thereof (such as a hydrochloride salt). In a convenient embodiment, the buffer components are histidine and histidine hydrochloride.

[0055] In an embodiment, the buffer components are present at a concentration of about 5 to 50 mM. It will be understood that the concentration of buffer components refers to the total concentration of all the buffer components, such as the total concentration of a weak alkali and its conjugate salt. In an embodiment, the buffer components are present at a concentration of about 10 to 50 mM, such as about 10 to 40 mM, or about 10 to 30 mM. Conveniently, the buffer components are present at a concentration of about 12 to 28 mM, such as about 14 to 26 mM, about 16 to 24 mM, or about 18 to 22 mM. More conveniently, the buffer components are present at a concentration of about 20 mM.

[0056] In an embodiment, the pharmaceutical composition according to the first aspect further comprises a surfactant. Suitable surfactants include PEG-35 castor oil (Cremophor® EL, Kolliphor® EL), PEG-40 hydrogenated castor oil (Cremophor® RH40, Kolliphor® RH40), D-alpha-tocopheryl PEG-1000 succinate (Vitamin E TPGS), caprylocaproyl PEG-8 glycerides (Labrasol®), PEG-32 glyceryl laureate (Gelucire® 44/14), poloxamers, and polysorbates. In a convenient embodiment, the surfactant is a polysorbate. In a convenient embodiment, the surfactant is PEG-60 sorbitan monostearate (polysorbate 60; Tween® 60), PEG-80 sorbitan monooleate (polysorbate 80; Tween® 80; Kolliphor® PS 80), or PEG-20 sorbitan monolaurate (polysorbate 20; Tween® 20). In a convenient embodiment the surfactant is polysorbate 80 or polysorbate 20. In terms of minimising insoluble particle formation in the compositions, polysorbate 80 was found to perform slightly better than polysorbate 20. Therefore, in a more convenient embodiment, the surfactant is polysorbate 80.

[0057] In an embodiment, the pharmaceutical composition according to the first aspect comprises about 0.01 to about 0.2 % w/v of surfactant. Conveniently, the composition comprises about 0.01 to about 0.1 % w/v of surfactant, more conveniently about 0.02 to about 0.05 % w/v of surfactant, yet more conveniently about 0.03 % w/v of surfactant. Conveniently, the composition comprises about 0.01 to about 0.1 % w/v of polysorbate surfactant (such as polysorbate 80), more conveniently about 0.02 to about 0.05 % w/v of polysorbate surfactant (such as polysorbate 80), yet more conveniently about 0.03 % w/v of polysorbate surfactant (such as polysorbate 80).

[0058] In an embodiment, the pharmaceutical composition according to the first aspect further comprises a stabiliser. Suitable stabilisers include carbohydrate-based stabilisers, amino acid-based stabilisers (e.g. arginine, histidine, lysine, proline, taurine or glycine), synthetic amphiphilic polymers (e.g. polyethylene glycols, or polypropylene glycols) and ionic liquids (e.g. ammonium-based ionic liquids or imidazolium-based salts). In an embodiment, the stabiliser is a carbohydrate-based stabiliser. Conveniently, the carbohydrate-based stabiliser is selected from the group including trehalose, mannitol, sucrose, maltose, lactose, fructose, xylitol, arabinol, erythritol, sylitol, sorbitol, raffinose, lactitol, maltitol, and inositol. In an embodiment, the stabiliser is a non-reducing sugar. Conveniently, the non-reducing sugar is selected from sucrose or trehalose. In terms of minimising insoluble particle formation in the compositions, sucrose was found to perform slightly better than trehalose. Therefore, in a more convenient embodiment, the stabiliser is sucrose.

[0059] In an embodiment, the pharmaceutical composition according to the first aspect comprises about 0.5 to about 10 % w/v of the stabiliser. Conveniently, the composition comprises about 1 to about 10 % w/v of stabiliser, such as about 2 to about 10 % w/v, about 2 to about 9 % w/v, or about 3 to about 9 % w/v. More conveniently, the composition comprises about 3 to about 8 % w/v of stabiliser, about 4 to about 8 % w/v of stabiliser, about 5 to about 7 % w/v of stabiliser, or yet more conveniently about 6 % w/v of stabiliser. More conveniently, the composition comprises about 3 to about 8 % w/v of non-reducing sugar stabiliser (such as sucrose), about 4 to about 8 % w/v of non-reducing sugar stabiliser (such as sucrose), about 5 to about 7 % w/v of non-reducing sugar stabiliser (such as sucrose), or yet more conveniently, about 6 % w/v of non-reducing sugar stabiliser (such as sucrose).

[0060] In an embodiment, the pharmaceutical composition according to the first aspect further comprises one or more pharmaceutically acceptable excipients. In an embodiment, the one or more pharmaceutically acceptable excipients are selected from a tonicity modifier, an antioxidant and a diluent. Tonicity modifiers may include sodium chloride, dextrose, sucrose, trehalose, mannitol, glycerol, glycerin, sorbitol, and mixtures thereof. Antioxidants may include ethylenediamine tetraacetic acid (EDTA), diethylenetriamine pentaacetate (DTPA), methionine, sodium bisulfite, sodium metabisulfite, monothioglycerol, ascorbic acid, sodium ascorbate, and mixtures thereof.

[0061] In an embodiment, the pharmaceutical composition according to the first aspect comprises the ADC and a buffer, wherein the pharmaceutical composition is a liquid and has a pH of about 5.4 to 6.5;

wherein the buffer is a histidine buffer and the composition further comprises a polysorbate surfactant and a non-reducing sugar stabiliser.

[0062] In a convenient embodiment, the pharmaceutical composition according to the first aspect comprises the ADC and a buffer, wherein the pharmaceutical composition is a liquid and has a pH of about 5.4 to 6.5; wherein the buffer is a histidine buffer and the composition further comprises polysorbate 20 or polysorbate 80 as a surfactant, and sucrose or trehalose as a stabiliser.

[0063] In a convenient embodiment, the pharmaceutical composition according to the first aspect comprises the ADC, a pH 5.4 to 6.5 histidine buffer, 0.01 to 0.2 % w/v of polysorbate 20 or polysorbate 80, and 0.5 to 10 % w/v of sucrose or trehalose. In a convenient embodiment, the pharmaceutical composition according to the first aspect comprises the ADC at a concentration of 5 to 100 mg/mL, a pH 5.4 to 6.5 histidine buffer, 0.01 to 0.2 % w/v of polysorbate 20 or polysorbate 80, and 0.5 to 10 % w/v of sucrose or trehalose. In a convenient embodiment, the pharmaceutical composition according to the first aspect comprises the ADC at a concentration of 5 to 50 mg/mL, a pH 6.0 to 6.2 histidine buffer at a concentration of 5 to 50 mM, 0.02 to 0.05 % w/v of polysorbate 20 or polysorbate 80, and 3 to 8 % w/v of sucrose or trehalose. In a more convenient embodiment, the pharmaceutical composition according to the first aspect comprises the ADC at a concentration of 15 to 25 mg/mL, a pH 6.0 to 6.2 histidine buffer at a concentration of 10 to 30 mM, 0.02 to 0.05 % w/v polysorbate 80, and 3 to 8 % w/v sucrose. In a yet more convenient embodiment, the pharmaceutical composition according to the first aspect comprises the ADC at a concentration of 18 to 22 mg/mL, a pH 6.1 histidine buffer at a concentration of 15 to 25 mM, 0.02 to 0.04 % w/v polysorbate 80, and 5 to 7 % w/v sucrose. In a yet even more convenient embodiment, the pharmaceutical composition according to the first aspect comprises the ADC at a concentration of 20 mg/mL, a pH 6.1 histidine buffer at a concentration of 20 mM, 0.03 % w/v polysorbate 80, and 6 % w/v sucrose.

[0064] In an embodiment, the pharmaceutical composition according to the first aspect has an osmolality of between about 150 and 450 mOsm/kg, such as about 200 and 400 mOsm/kg or such as between about 200 and 300 mOsm/kg.

[0065] In an embodiment, in the pharmaceutical composition according to the first aspect the ADC is physically and/or chemically stable within the pharmaceutical composition for at least four weeks when stored at 2-8°C or -20°C.

[0066] In this context, physical stability relates to maintenance of the appearance of the liquid composition, and the dissolution of ADC in the liquid composition being maintained over the storage period referred to. The appearance may be compared by visual inspection of colour and the presence of any visible particulate matter.

[0067] Chemical stability relates to low levels of impurities being formed over the storage period referred to. Typically, these are impurities related to the ADC and may be measured by suitable techniques such as SEC, non-reducing CE-SDS, HIC, or HPLC.

[0068] Aggregate content may be measured by SEC-HPLC. In an embodiment, the pharmaceutical composition contains less than 5% aggregates (such as less than 4%, or less than 3% aggregates) as determined by SEC-HPLC when stored at 2-8°C or -20°C for at least four weeks.

[0069] The content of acidic and/or alkaline components in the composition may be determined by CEX-HPLC. In an embodiment, the content of acidic peaks in the pharmaceutical composition increases by less than 5% (such as less than 4%, less than 3%, less than 2%, or less than 1%) as determined by CEX-HPLC when stored at 2-8°C or -20°C for at least four weeks. In an embodiment, the content of alkaline peaks in the pharmaceutical composition increases by less than 5% (such as less than 4%, less than 3%, less than 2%, or less than 1%) as determined by CEX-HPLC when stored at 2-8°C or -20°C for at least four weeks. In an embodiment, the content of acidic peaks in the pharmaceutical composition increases by less than 10% (such as less than 9%, less than 8%, less than 7%, or less than 6%) as determined by CEX-HPLC when stored at 25°C for at least three months. In an embodiment, the content of alkaline peaks in the pharmaceutical composition increases by less than 12% (such as less than 11%, less than 10%, less than 9%, or less than 8%) as determined by CEX-HPLC when stored at 25°C for at least three months.

[0070] In an embodiment, the purity of the pharmaceutical composition is greater than 96% as determined by non-reducing CE-SDS when stored at 2-8°C or -20°C for at least four weeks. In an embodiment, the purity of the pharmaceutical composition is greater than 95% (such as greater than 96%) as determined by non-reducing CE-SDS when stored at 25°C for at least three months.

[0071] In an embodiment, the content of free drug in the pharmaceutical composition is less than 0.5 mg/ml (such as less than 0.4 mg/ml, or less than 0.3 mg/ml) as determined by RP-HPLC when stored at 2-8°C or -20°C for at least four weeks. In an embodiment, the content of free drug in the pharmaceutical composition is less than 0.75 mg/ml (such as less than 0.6 mg/ml, or less than 0.5 mg/ml) as determined by RP-HPLC when stored at 25°C for at least four weeks.

[0072] In an embodiment, the pharmaceutical composition is in the form of an injectable aqueous solution. Conveniently, the injectable solution is sterile, isotonic, free from pyrogenic contamination, and free from visible particulate matter. In an embodiment, the injectable aqueous solution is for parenteral administration by intravenous infusion.

[0073] In an embodiment, the pharmaceutical composition according to the first aspect is in the form of an injectable aqueous solution for administration by intravenous infusion and the solution is substantially free from visible particulate matter. Conveniently, the solution is free from visible particulate matter.

Process for preparing the pharmaceutical compositions

[0074] According to a second aspect of the invention, there is provided a process for manufacturing a pharmaceutical composition according to the first aspect. The process comprises the steps of mixing together the ADC and the buffer; and optionally any one or more additional components, optionally in any amount, concentration, or form; and optionally adjusting any one or more parameters, such as pH, in relation to the pharmaceutical composition.

[0075] In an embodiment, the process comprises the steps of (i) mixing together the ADC and buffer components; (ii) adding the liquid (e.g. water) to give the desired concentration; (iii) adjusting the pH if necessary to ensure it is within the range 5.4 to 6.5; and (iv) optionally adding one or more pharmaceutically acceptable additional excipients selected from a surfactant, a stabiliser, a tonicity modifier, an antioxidant and a diluent.

[0076] In an embodiment, the process comprises the steps of (i) dissolving the buffer components in the liquid (e.g. water) to give the desired concentration; (ii) adjusting the pH of the buffer solution if necessary to ensure it is within the range 5.4 to 6.5; (iii) adding the ADC to the buffer solution; and (iv) optionally adding one or more pharmaceutically acceptable additional excipients selected from a surfactant, a stabiliser, a tonicity modifier, an antioxidant and a diluent.

[0077] In a convenient embodiment, the process comprises dialysis. Therefore, in an embodiment, the process comprises the steps of (i) dissolving the buffer components in the liquid (e.g. water) to give the desired concentration, and optionally adding a stabiliser to the buffer solution; (ii) adjusting the pH of the buffer solution if necessary to ensure it is within the range 5.4 to 6.5; (iii) adding a dialysis bag containing the ADC to the buffer solution; (iv) carrying out dialysis with one or more changes of the buffer solution; and (v) optionally adding to the resultant composition one or more pharmaceutically acceptable additional excipients selected from a surfactant, a tonicity modifier, an antioxidant and a diluent.

[0078] In an embodiment, the process comprises the steps of (i) dissolving the buffer components in the liquid (e.g. water) to give the desired concentration, and adding a stabiliser selected from sucrose and trehalose to the buffer solution; (ii) adjusting the pH of the buffer solution if necessary to ensure it is within the range 5.4 to 6.5; (iii) adding a dialysis bag containing the ADC to the buffer solution; (iv) carrying out dialysis with one or more changes of the buffer solution; and (v) adding to the resultant composition surfactant selected from polysorbate 20 and polysorbate 80, and optionally adding one or more pharmaceutically acceptable additional excipients selected from a tonicity modifier, an antioxidant and a diluent.

[0079] Other suitable processes may be used to manufacture the pharmaceutical compositions, as will be apparent to a person skilled in the art, such as processes employing ultrafiltration, diafiltration, or tangential flow filtration.

[0080] In an embodiment, there is provided a product obtainable by, or obtained by, the process of manufacturing a pharmaceutical composition according to the second aspect.

Lyophilized Formulation

[0081] According to a third aspect of the invention, there is provided a lyophilized formulation comprising the ADC and a buffer, wherein the formulation can be reconstituted to form the pharmaceutical composition according to the first aspect. Advantageously the composition may be stored as a dried, lyophilized formulation, prior to reconstitution as a liquid immediately prior to use

(e.g. for intravenous infusion). In an embodiment, the lyophilized formulation further comprises a stabilizer and/or a surfactant and optionally one or more pharmaceutically acceptable excipients, such as a tonicity modifier, an antioxidant, or a bulking agent. The bulking agent may include mannitol, sorbitol, glucose, glycine, hydroxyethyl starch, polyvinyl pyrrolidone (PVP), or a mixture thereof. Conveniently, the lyophilized formulation is a sterile powder.

[0082] In a fourth aspect of the invention, there is provided a process for manufacturing a lyophilized according to the third aspect. The process comprises the step of lyophilizing or freeze-drying the pharmaceutical composition according to the first aspect.

Container

[0083] According to a fifth aspect of the invention, there is provided a container comprising a pharmaceutical composition according to the first aspect, or a lyophilized formulation according to the third aspect, wherein the container can be a vial, single-use vial, light-protected vial, ampoule, syringe, pre-filled syringe, injection pen or intravenous infusion bag.

[0084] In an embodiment, the container is a transparent, amber or brown container. Conveniently the container is a vial, ampoule, or syringe made from glass; conveniently the glass is borosilicate glass. Conveniently the container is made from plastic; conveniently the plastic is polyethylene, polypropylene, a polyolefin, polyethylene terephthalate, polyethylene vinyl acetate, or polyvinyl chloride.

[0085] In an embodiment, there is provided a container comprising a pharmaceutical composition according to the first aspect, wherein the container is a vial, single-use vial, light-protected vial, ampoule, syringe, pre-filled syringe, injection pen, or intravenous infusion bag. In an embodiment, there is provided a container comprising a lyophilized formulation according to the third aspect, wherein the container is a vial, single-use vial, light-protected vial, or ampoule.

Therapeutic Uses

[0086] According to a sixth aspect of the invention, there is provided a pharmaceutical composition according to the first aspect, or a lyophilized formulation according to the third aspect, for use as a medicament.

[0087] In an embodiment, there is provided a pharmaceutical composition according to the first aspect, or a lyophilized formulation according to the third aspect, for use in the treatment of cancer, or an autoimmune disease.

[0088] In an embodiment, there is provided a pharmaceutical composition according to the first aspect, or a lyophilized formulation according to the third aspect, for use in the treatment of cancer. In an embodiment, the cancer is a TROP2-associated tumour. In an embodiment, the TROP2-associated tumour includes a tumour overexpressing TROP2 (e.g. a tumour marked as ++ or +++ using immunohistochemistry) or a tumour with one or more TROP2 gene mutations. In an embodiment, the cancer is selected from fibrosarcoma, myosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendothelial sarcoma, synovium, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon cancer, pancreatic cancer, breast cancer, thyroid cancer, endometrial cancer, melanoma, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous carcinoma, papillary carcinoma, papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, bronchial carcinoma, renal cell carcinoma, liver cancer, bile duct cancer, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung cancer, small cell lung cancer, bladder cancer, epithelial cancer, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma and retinoblastoma. Conveniently, the cancer is selected from breast cancer, gastric cancer, lung cancer, ovarian cancer, and urothelial cancer.

[0089] In an embodiment, there is provided the use of a pharmaceutical composition according to the first aspect, or a lyophilized formulation according to the third aspect, in the manufacture of a medicament for the treatment of cancer, or an autoimmune disease. In an embodiment, there is provided the use of a pharmaceutical composition according to the first aspect, or a lyophilized formulation according to the third aspect, in the manufacture of a medicament for the treatment of cancer. In an embodiment, the cancer is a TROP2-associated tumour. In an embodiment, the TROP2-associated tumour includes a tumour overexpressing TROP2 or a tumour with one or more TROP2 gene mutations. In an embodiment, the cancer is selected from fibrosarcoma, myosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma,

lymphangiosarcoma, lymphangioendothelial sarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon cancer, pancreatic cancer, breast cancer, thyroid cancer, endometrial cancer, melanoma, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous carcinoma, papillary carcinoma, papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, bronchial carcinoma, renal cell carcinoma, liver cancer, bile duct cancer, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung cancer, small cell lung cancer, bladder cancer, epithelial cancer, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma and retinoblastoma. Conveniently, the cancer is selected from breast cancer, gastric cancer, lung cancer, ovarian cancer, and urothelial cancer.

[0090] In an embodiment, there is provided a method of treating cancer, or an autoimmune disease, in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition according to the first aspect, or a lyophilized formulation according to the third aspect. In an embodiment, there is provided a method of treating cancer, in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition according to the first aspect, or a lyophilized formulation according to the third aspect. In an embodiment, there is provided a method of treating cancer, or an autoimmune disease, in a subject in need thereof, the method comprising the steps of (i) reconstituting a lyophilized formulation according to the third aspect; and (ii) administering to the subject a therapeutically effective amount of the reconstituted formulation from step (i). In an embodiment, the cancer is a TROP2-associated tumour. In an embodiment, the TROP2-associated tumour includes a tumour overexpressing TROP2 or a tumour with one or more TROP2 gene mutations. In an embodiment, the cancer is selected from fibrosarcoma, myosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendothelial sarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon cancer, pancreatic cancer, breast cancer, thyroid cancer, endometrial cancer, melanoma, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous carcinoma, papillary carcinoma, papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, bronchial carcinoma, renal cell carcinoma, liver cancer, bile duct cancer, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung cancer, small cell lung cancer, bladder cancer, epithelial cancer, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma and retinoblastoma. Conveniently, the cancer is selected from breast cancer, gastric cancer, lung cancer, ovarian cancer, and urothelial cancer. Conveniently, the subject is a mammal; more conveniently the subject is a human.

Routes of Administration

[0091] The method of treatment may comprise administering a pharmaceutical composition as disclosed herein, to a subject by any convenient route of administration.

[0092] The route of administration for the pharmaceutical compositions according to the present invention may be parenteral, for example, by injection or infusion, including subcutaneous, intradermal, intramuscular, intravenous, intraarterial, intracardiac, intrathecal, intraspinal, intracapsular, subcapsular, intraorbital, intraperitoneal, intratracheal, subcuticular, intraarticular, subarachnoid, and intrasternal; or by implant of a depot or reservoir, for example, subcutaneously or intramuscularly. Conveniently, the route of administration for the pharmaceutical compositions according to the present invention is intravenous infusion.

Dosages

[0093] The method of treatment typically comprises administering a therapeutically effective amount of a pharmaceutical composition according to the present invention, to a subject.

[0094] Appropriate dosages of the pharmaceutical compositions according to the present invention, can vary from patient to patient. Determining the optimal dosage will generally involve balancing the level of therapeutic benefit against any risk or deleterious side effects. The selected dosage level will depend on a variety of factors including, but not limited to, the route of administration, the time of administration, the rate of excretion of the compound, the duration of the treatment, other active agents,

compounds, and/or materials used in combination, the severity of the condition, and the species, sex, age, weight, condition, general health, and prior medical history of the patient. The dosage and route of administration will ultimately be at the discretion of the clinician, although generally the dosage will be selected to achieve concentrations at the site of action which achieve the desired effect without causing substantial harmful or deleterious side-effects.

[0095] Administration can be effected in one dose, continuously or intermittently (e.g., in divided doses at appropriate intervals) throughout the course of treatment. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating clinician.

Kit of Parts

[0096] According to an eighth aspect of the invention, there is provided a kit of parts comprising a pharmaceutical composition according to the first aspect, or the lyophilized formulation according to the third aspect, in a container and, optionally, a set of instructions with directions regarding the administration (e.g. by way of intravenous infusion) of the pharmaceutical composition or the lyophilized formulation.

[0097] In an embodiment, there is provided a kit of parts comprising a lyophilized formulation according to the third aspect in a container, a diluent for reconstituting the lyophilized formulation and, optionally, a set of instructions with directions regarding the administration (e.g. by way of intravenous infusion) of the reconstituted formulation. In a convenient embodiment, the diluent is an aqueous sodium chloride solution. In a convenient embodiment, the diluent is sterile water. In a convenient embodiment, the diluent is bacteriostatic water.

EXAMPLES

General Methods

Protein concentration (UV method)

[0098] Protein concentration was measured using a UV-visible spectrophotometer. A Nanodrop UV spectrophotometer was used, and ultrapure water was used for blank correction. After mixing the test sample, 2.5 μ L of the sample was pipetted and its absorbance at 280 nm was measured to determine the sample protein concentration.

Purity (SEC-HPLC)

[0099] Sample purity was tested by size exclusion high-performance liquid chromatography (SEC-HPLC). The chromatographic column was TSKgel G3000SWXL, a 7.8 $\times$ 300mm gel chromatography column; the mobile phase was 0.1 mol/L potassium dihydrogen phosphate-0.125 mol/L potassium chloride solution, pH 7.2. The test sample containing approximately 5 mg of sample was diluted with ultrapure water to 1 mL. Detailed chromatographic conditions and parameters are shown in the table below. The percentages of monomers, aggregates, and fragments were calculated using the area normalization method.

Conditions	Parameters
Detector wavelength	280 nm
Column temperature	30°C
Sample chamber temperature	8°C
Flow rate	0.50 mL/min
Injection volume	20 μ L
Running time	30.0 min

Charge variant (CEX)

[00100] Sample purity was tested by cation exchange high-performance liquid chromatography (CEX-HPLC). The chromatographic column was YMC BioPro IEX SF (4.6*100 mm) cation exchange chromatography column. The mobile phase composition was A: 40 mM (N-morpholino)ethanesulfonic acid (MES) (pH 6.0), B: 40 mM MES + 100 mM NaCl (pH 6.0). The test sample was diluted with ultrapure water to approximately 2 mg per 1 mL. The chromatographic parameters were set as follows: column temperature was 45°C, sample chamber temperature was 8°C, flow rate was 0.8 mL/min, injection volume was 50 μ L, detection wavelength was 280 nm, elution time was 40 minutes. The elution gradient is shown in the table below. The area normalization method was used to calculate the relative percentages of acidic components, main peaks and basic components.

Time	A%	B%
0.00	90	10
2.00	90	10
8.00	60	40
30.00	0	100
35.00	0	100
35.10	90	10
40.00	90	10

Purity (R CE-SDS)

[00101] Purity by R-CE-SDS was detected using a capillary electrophoresis instrument (Beckman Coulte PA800 Plus). The test sample was diluted with ultrapure water to 10 mg/ml, and to 10 μ L of the diluted sample solution was added 85 μ L of pH 6.2 sample buffer (citric acid-phosphate buffer), and 5 μ L of β -mercaptoethanol, and the sample was vortex-mixed. After mixing, and heating at 70°C for 5 minutes, the sample was cooled to room temperature, and centrifuged at 13,000 rpm for 10 minutes before loading. The peak area percentages of LC, LC-Drug, and HC were calculated using the corrected peak area. The sum of the three percentages is the reduction purity. Instrument parameters are shown in the table below:

Chromatographic conditions	Parameters
Detector wavelength	200 nm
Capillary column temperature	25°C
Sample chamber temperature	15°C
Detector	PDA
Detection window width	200 μ m
Detection wavelength	220 nm
Data collection frequency	2 Hz
Peak width	16~25
Detection filtering mode	Normal

Purity (NR CE-SDS)

[00102] Purity by NR-CE-SDS was detected using a capillary electrophoresis instrument (Beckman Coulte PA800 Plus). The test sample was diluted with ultrapure water to 10 mg/ml, and to 10 μ L of the diluted sample solution was added 85 μ L of pH 6.2 sample buffer (citric acid-phosphate buffer), and 5 μ L of 500 mmol/L iodoacetamide (IAM), and the sample was vortex-mixed. After homogenization, and heating at 70°C for 5 minutes, the sample was cooled to room temperature, and centrifuged at 13,000 rpm for 10 minutes before loading. The purity of the main peak was calculated as the percentage of the calibrated area of the IgG main peak to the total calibrated peak area. The instrument parameters are shown in the table below:

Chromatographic conditions	Parameters
Detection window width	200 μ m
Detector wavelength	220 nm
Capillary column temperature	25°C
Sample chamber temperature	15°C
Detector	PDA
Data collection frequency	2 Hz
Peak width	16~25
Detection filtering mode	Normal

DAR Value

[00103] Hydrophobic interaction chromatography (HIC) was used to detect the drug-antibody ratio (DAR) value. The chromatographic column was Sepex Proteomix HIC Butyl NP5, 4.6 $\times$ 35 mm, particle

size 5 μm . The composition of mobile phase A was 25 mM PB + 2 M ammonium sulfate, pH 7.0; the composition of mobile phase B was 25 mM PB: IPA=70:30 (v:v), pH 7.0 $\pm$ 0.05. The test sample was diluted with pure water to approximately 5 mg per 1 mL. The chromatographic parameters were set as follows: column temperature was 35°C, sample chamber temperature was 5 $\pm$ 3°C, flow rate was 0.8 mL/min, injection volume was 5 μL , detection wavelength was 280 nm, elution time was 20 minutes. The sample DAR0, DAR2, and DAR4 peak area percentages were calculated through the area normalization method, and finally the DAR value of the test product was calculated. The elution gradient was as follows:

Time (minutes)	Phase A (%)	Phase B (%)
0.0	80.0	20.0
2.0	80.0	20.0
15.0	10.0	90.0
15.1	0	100.0
17.0	0	100.0
17.1	80.0	20.0
20.0	80.0	20.0

Free Drug (RP-HPLC)

[00104] Free Drug detection was performed by reversed-phase high-performance liquid chromatography (RP-HPLC). The chromatographic column was Waters Xbridge C18, 4.6 $\times$ 150 mm, particle size 3.5 μm . Mobile phase A was 20 mM KH_2PO_4 solution (pH 5.0), and mobile phase B was acetonitrile solution. The protein was precipitated using acetone aqueous solution and the supernatant was taken for Free Drug determination. The chromatographic conditions were set as follows: the flow rate was 0.8 mL/min, the injection volume was 10 μL , the column temperature was 25°C, the sample chamber temperature was 4°C, the detection wavelength was 264 nm, and the analysis time was 20 min. The content in the sample was calculated by use of an external standard. The elution gradient was as follows:

Time (minutes)	Phase A (%)	Phase B (%)
0.0	75	25
1.0	75	25
10.0	35	65
12.0	20	80
15.0	20	80
15.1	75	25
20.0	75	25

Binding Activity (ELISA)

[00105] Binding activity was determined by enzyme-linked immunosorbent assay (ELISA). Antigen (GR1002T) was added to the enzyme plate, it was coated at 2-8°C overnight, and then blocked with blocking solution. After washing the plate with washing liquid, the reference product and test solution were added respectively, and the plate was incubated with shaking at 25°C for 1 hour. After washing the plate with washing liquid, horseradish peroxidase-labelled Goat anti-human IgG-Fc antibody solution was added and the enzyme plate was incubated with shaking at 25°C for 1 hour. After washing the plate with washing solution, TMB was added for color development and to protect from light. After 10 minutes the reaction was stopped with stop solution, and the absorbance was measured at a wavelength of 450 nm. OD_{450} was measured with a microplate reader and the software tool that came with the microplate reader was used to create a four-parameter Logistic curve ($Y = (A-D)/(1+(X/C)^B) + D$) with the concentration as the abscissa and the OD_{450} value as the ordinate. After fitting a standard curve was developed, to obtain the curve fitting correlation coefficient R^2 and the EC_{50} of the standard and sample, in order to calculate the relative binding activity of the sample. The calculation formula was as follows: relative binding activity of sample (%) = standard $\text{EC}_{50} \div$ sample $\text{EC}_{50} \times 100\%$.

Appearance and visible foreign matter (visual method)

[00106] Appearance of the samples was assessed by visual inspection. The light intensity of the clarity detector was maintained between 1000 lx and 1500 lx. The sample was held at eye level and gently shaken or inverted to prevent air bubbles. Visual inspection was performed in front of black and white backgrounds. The results were recorded in terms of appearance and visible foreign matter.

Insoluble Particles (photoresist method)

[00107] Insoluble particles were detected through the AccuSizer A2000 insoluble particle detector. The parameters were set according to the table below.

Injection volume	200 μ L
Testing frequency	4
Flow rate	10 ml/min
Sensitivity mode	Extinction
Background values	50 counts/mL

Osmolality

[00108] Detection was carried out using an Osmomat 3000 osmotic pressure detector. Before sample detection, a calibration solution (300 mOsm/L, 500 mOsm/L) was used to calibrate the detector.

Materials

Reagent name	Manufacturer	Batch number
Acetic acid	Southern Examination	19103026613
Sodium acetate trihydrate	Hunan Erkang	200918216B
Histidine hydrochloride monohydrate	Merck	K52851254 050
Histidine	Merck	K52760952 048
Citric acid	Hunan Huari	20200106
Sodium citrate	Hunan Huari	20200204
Succinic acid	Merck	K53911781 202
Sodium hydroxide	Merck	MB1937820 119
Sucrose	Pfanstiehl	36697A
Trehalose dihydrate	Pfanstiehl	36602A
Mannitol	Merck	MP19055796 017
Arginine hydrochloride	Shanghai Concorde	20220913
Sodium chloride	Jiangsu Diligence	20200913
Polysorbate 20	Croda	0001694815
Polysorbate 80	Croda	0001494588

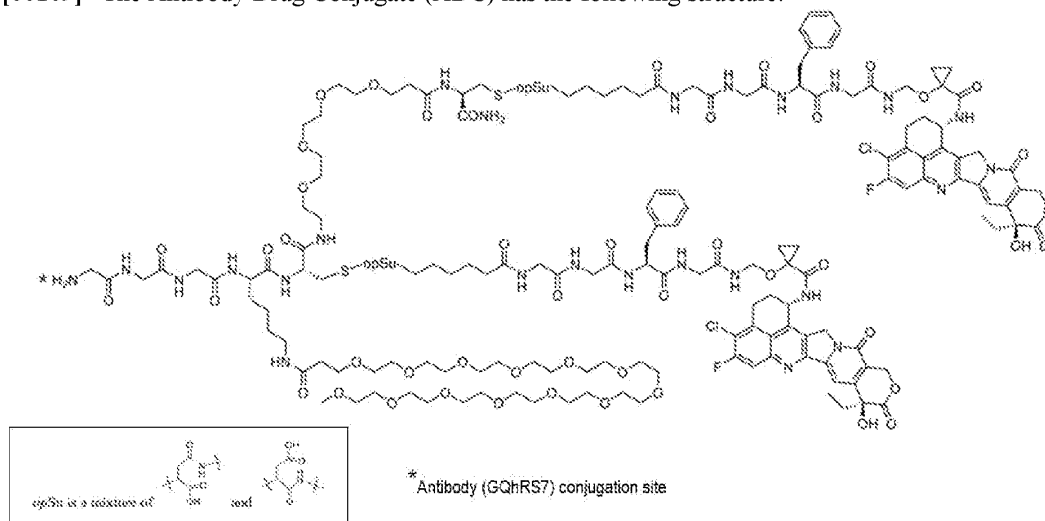
Abbreviations

DCC	N,N'-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DIC	N,N'-Diisopropylcarbodiimide
DIEA	Diethylamine
DMF	Dimethyl formamide
EA	Ethyl Acetate
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
Et	Ethyl
FCC	Flask column chromatography
HATU	Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium
HOAt	1-Hydroxy-7-azabenzotriazole
HOBt	1-Hydroxybenzotriazole
Me	Methyl
NCS	N-Chlorosuccinimide
PE	Petroleum ether
THF	Tetrahydrofuran
TFA	Trifluoroacetic acid
TIS	Triisopropylsilane
TLC	Thin layer chromatography
TSTU	N,N,N',N'-Tetramethyl-O-(N-succinimidyl)uronium tetrafluoroborate
W	Week
D	Day
H	Hour
I	Illumination
S	Shake
F/T	Freeze/Thaw
C	Cycle
RT	Room Temperature
SEC	Size Exclusion Chromatography

iCIEF	imaged Capillary Isoelectric Focusing
HPLC	High Performance Liquid Chromatography
NR CE-SDS	Non-reduced Capillary Electrophoresis Sodium Dodecylsulfate
R CE-SDS	Reduced Capillary Electrophoresis Sodium Dodecylsulfate
DAR Value	Drug-to-antibody Ratio Value
HIC	Hydrophobic Interaction Chromatography
N/A	Not Applicable
ND	Not Detected

Example 1: Formation of Antibody Drug Conjugate (ADC)

[00109] The Antibody Drug Conjugate (ADC) has the following structure:



1.1 Preparation of Payload – Scheme A

Scheme A: Synthetic Route to Payload

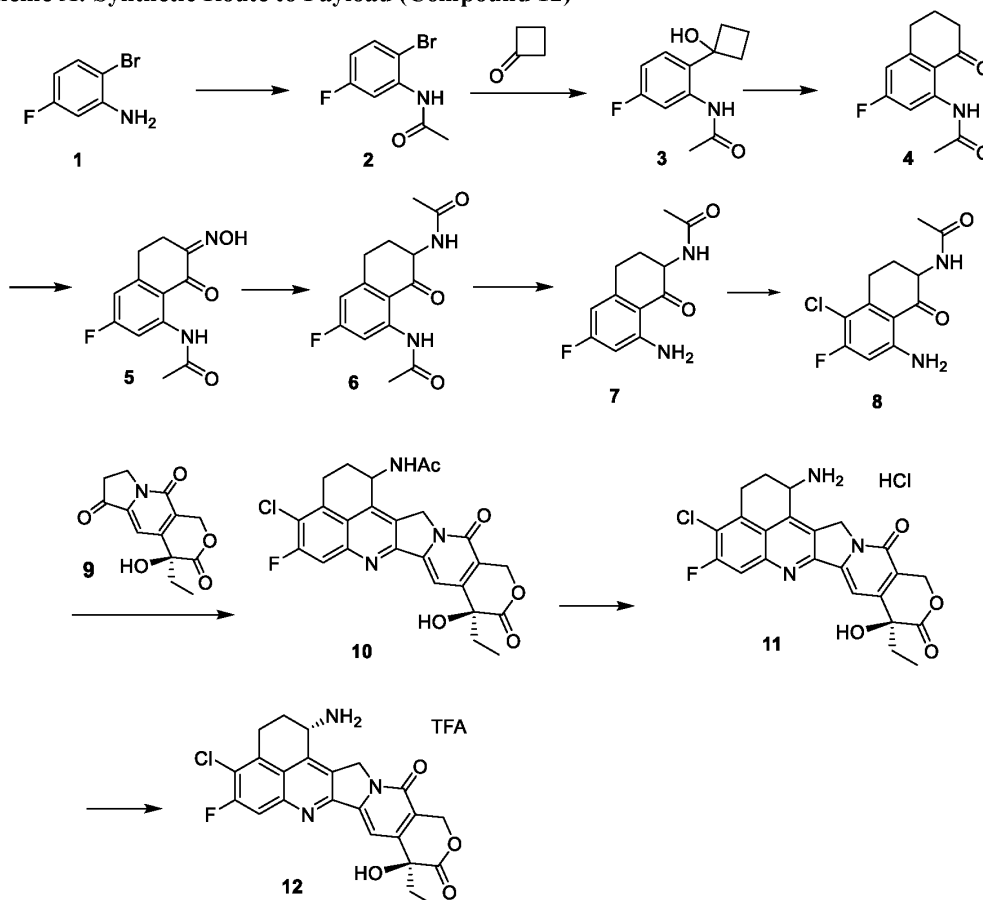
1.1.1 Formation of N-(2-bromo-5-fluorophenyl)acetamide (Compound 2)

[00110] To a stirred solution of acetic anhydride (214 g, 2.10 mol) in acetic acid (500 mL) was added conc. H_2SO_4 (3 mL), followed with 2-bromo-5-fluoroaniline – Compound 1 (100 g, 526.27 mmol) in portions at room temperature. The mixture was stirred for 3 h, then poured into 2000 mL ice-water. A precipitate was formed, which was collected by filtration and dried in vacuo at room temperature to afford N-(2-bromo-5-fluorophenyl)acetamide (105 g) as a yellow solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.68 (dd, $J = 8.9, 6.0$ Hz, 1H), 7.61 (ddd, $J = 10.7, 5.3, 3.1$ Hz, 1H), 7.02 (ddd, $J = 8.9, 8.0, 3.1$ Hz, 1H), 2.11 (s, 3H). MS m/z 232.0(M+H).

1.1.2 Formation of N-(5-fluoro-2-(1-hydroxycyclobutyl)phenyl)acetamide (Compound 3)

[00111] To a stirred solution of Compound 2 (105 g, 452.48 mmol) in THF (1000 mL) was added n-BuLi (594 mL, 1.6 M in n-hexane, 950.22 mmol) dropwise over 1 h at -78°C . After completion, the mixture was stirred for 0.5 h under N_2 . Then a solution of cyclobutanone (38.06 g, 542.98 mmol) in THF (50 mL) was added dropwise at -78°C over 0.5 h, the mixture was stirred at -78°C to room temperature for 6 h. The mixture was poured into 500 mL saturated NH_4Cl aq at 0°C , extracted with ethyl acetate (500 mL x 3), washed with brine (250 mL x 2), dried over Na_2SO_4 and concentrated. The mixture was triturated with (PE/EA = 1:1, 100 mL) for 10 mins, filtered and the cake was collected and dried in vacuo to afford N-(5-fluoro-2-(1-hydroxycyclobutyl)phenyl)acetamide (24 g) as a yellow solid. LCMS m/z 206.1(M-18+H), 246.1(M+Na).

Scheme A: Synthetic Route to Payload (Compound 12)



1.1.3 Formation of N-(3-fluoro-8-oxo-5,6,7,8-tetrahydronaphthalen-1-yl)acetamide (Compound 4)

[00112] To a stirred mixture of Compound 3 (24 g, 107.50 mmol) in CH_2Cl_2 (170 mL) and water (170 mL) was added silver nitrate (AgNO_3) (5.48 g, 32.25 mmol) and potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$) (58.12 g, 215.01 mmol), the mixture was stirred at 30 °C for 6 h. The mixture was filtered on Celite and washed with CH_2Cl_2 (100 mL), the filtrate was concentrated and purified by FCC (EA/PE=0-40%) to afford N-(3-fluoro-8-oxo-5,6,7,8-tetrahydronaphthalen-1-yl)acetamide (14 g) as a light yellow solid. MS m/z 222.1(M+H).

1.1.4 Formation of N-(3-fluoro-7-(hydroxyimino)-8-oxo-5,6,7,8-tetrahydronaphthalen-1-yl)acetamide (Compound 5)

[00113] To a stirring mixture Compound 4 (14 g, 63.28 mmol) in THF (500 mL) at 0 °C was added 1-butyl nitrite (8.48 g, 63.28 mmol), followed with t-BuOK (8.52 g, 75.94 mmol). The mixture was stirred at 0 °C for 2 h. After completion, the mixture was acidified by HCl (2 N) to adjust pH=3. The mixture was extracted by ethyl acetate (200 mL x 3), washed by brine (100 mL x 2), dried over Na_2SO_4 and concentrated under reduced pressure. The crude mixture was triturated with tert-butyl methyl ether (200 mL) for 10 mins, filtered and the cake was collected and dried in vacuo to afford N-(3-fluoro-7-(hydroxyimino)-8-oxo-5,6,7,8-tetrahydronaphthalen-1-yl)acetamide (12 g) as a yellow solid. MS m/z 251.1(M+H).

1.1.5 Formation of N,N'-(3-fluoro-8-oxo-5,6,7,8-tetrahydronaphthalene-1,7-diyl)diacetamide (Compound 6)

[00114] To a solution of Compound 5 (12 g, 47.96 mmol) in acetic anhydride (90 mL) and THF (90 mL) was added 10% Pd/C (1 g), the mixture was stirred at 25 °C under H_2 atmosphere for 16 h. After cooling to 0 °C, Et_3N (20 mL) was added dropwise, the mixture was stirred at 0 °C for 1 h. After filtering

through Celite®, the filtrate was poured into ice-water (500 mL), and extracted with ethyl acetate (500 mL x 3), washed with brine (250 mL x 2), dried over Na₂SO₄ and concentrated. The residue was triturated with tert-butyl methyl ether (120 mL) for 10 mins, filtered and the cake was collected and dried in vacuo to give N,N'-(3-fluoro-8-oxo-5,6,7,8-tetrahydronaphthalene-1,7-diyl)diacetamide (7.9 g) as a yellow solid. MS m/z 279.1(M+H).

1.1.6 Formation of N-(8-amino-6-fluoro-1-oxo-1,2,3,4-tetrahydronaphthalen-2-yl)acetamide (Compound 7)

[00115] To a solution of Compound 6 (7.9 g, 28.39 mmol) in MeOH (150 mL) was added HCl aq (2 N, 150 mL), the mixture was stirred at 50 °C for 7 h. After cooling to 0 °C, sat. NaHCO₃ aq was added dropwise to adjust pH = 8. The mixture was extracted with ethyl acetate (200 mL x 3), washed with brine (200 mL x 2), dried over Na₂SO₄ and concentrated under reduced pressure to give N-(8-amino-6-fluoro-1-oxo-1,2,3,4-tetrahydronaphthalen-2-yl)acetamide (6.0 g) as a yellow solid. ¹H NMR (400 MHz, Chloroform-d) δ 6.57 (s, 3H), 6.18 (td, J = 11.1, 2.4 Hz, 2H), 4.52 (dt, J = 13.3, 5.0 Hz, 1H), 3.13 (ddd, J = 17.5, 13.0, 4.6 Hz, 1H), 3.00 – 2.81 (m, 1H), 2.69 (dtd, J = 9.4, 4.6, 2.5 Hz, 1H), 2.09 (s, 3H), 1.79 (qd, J = 13.0, 4.3 Hz, 1H). MS m/z 237.1(M+H).

1.1.7 Formation of N-(8-amino-5-chloro-6-fluoro-1-oxo-1,2,3,4-tetrahydronaphthalen-2-yl)acetamide (Compound 8)

[00116] To a solution of Compound 7 (4.0 g, 16.93 mmol) in DMF (80 mL) was added NCS (2.26 g, 16.93 mmol) in portions at 0 °C, the mixture was stirred at room temperature for 16 h. The mixture was poured into 200 mL ice-water. A precipitate was formed, which was collected by filtration and dried in vacuo at room temperature to afford N-(8-amino-5-chloro-6-fluoro-1-oxo-1,2,3,4-tetrahydronaphthalen-2-yl)acetamide (4.0 g) as a yellow solid. ¹H NMR (400 MHz, DMSO-d₆) δ 8.11 (d, J = 8.0 Hz, 1H), 7.71 (s, 2H), 6.62 (d, J = 11.9 Hz, 1H), 4.53 (ddd, J = 13.0, 8.0, 4.7 Hz, 1H), 3.18 – 3.04 (m, 1H), 2.91 (ddd, J = 17.5, 12.4, 4.8 Hz, 1H), 2.21 – 2.08 (m, 1H), 1.99 – 1.83 (m, 4H). MS m/z 271.0 (M+H).

1.1.8 Formation of N-((9S)-4-chloro-9-ethyl-5-fluoro-9-hydroxy-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)acetamide (Compound 10)

[00117] To a mixture of Compound 8 (4.0 g, 14.78 mmol) in toluene (400 mL) was added (S)-4-ethyl-4-hydroxy-7,8-dihydro-1H-pyrano[3,4-f]indolizine-3,6,10(4H)-trione (4.28 g, 16.25 mmol, CAS No. 110351-94-5), pyridinium p-toluenesulfonate (1.11 g, 4.43 mmol) and o-cresol (10 mL), the mixture was heated to reflux under N₂ for 24 h. The solvent was removed by reduced pressure and the mixture was purified by FCC (THF/CH₂Cl₂=0-60%) to afford N-((9S)-4-chloro-9-ethyl-5-fluoro-9-hydroxy-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)acetamide (4.1 g) as a brown solid. MS m/z 498.1(M+H).

1.1.9 Formation of (9S)-1-amino-4-chloro-9-ethyl-5-fluoro-9-hydroxy-1,2,3,9,12,15-hexahydro-10H,13H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinoline-10,13-dione hydrochloride (Compound 11)

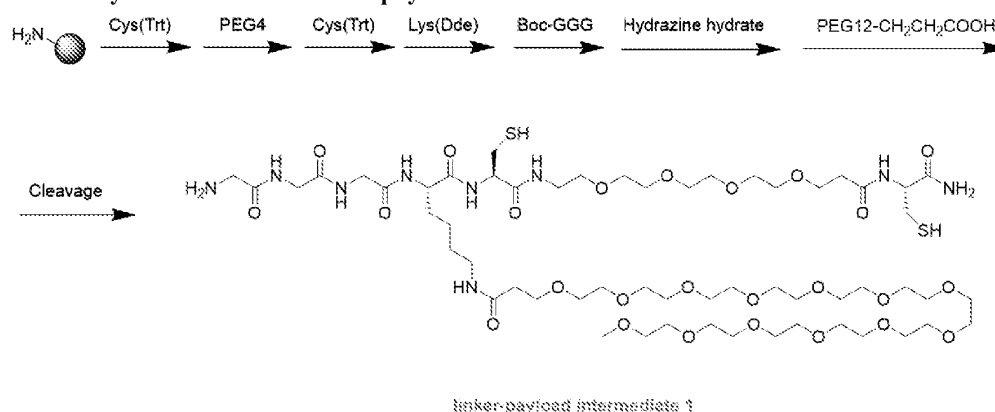
[00118] A mixture of Compound 10 (2.0 g, 4.02 mmol) in 20 mL conc. HCl was stirred at 70 °C under N₂ for 36 h. The mixture was concentrated under reduced pressure to give crude (9S)-1-amino-4-chloro-9-ethyl-5-fluoro-9-hydroxy-1,2,3,9,12,15-hexahydro-10H,13H-benzo[de]pyrano[3',4':6,7]-indolizino[1,2-b]quinoline-10,13-dione hydrochloride (2 g) as a brown solid. MS (ESI) m/z 456.1 (M+H).

1.1.10 Formation of (9S)-1-amino-4-chloro-9-ethyl-5-fluoro-9-hydroxy-1,2,3,9,12,15-hexahydro-10H,13H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinoline-10,13-dione trifluoroacetate (Compound 12)

[00119] Compound 12 was prepared by prep-HPLC from Compound 11. ¹H NMR (400 MHz, DMSO-d₆) δ 8.51 (d, J = 4.8 Hz, 3H), 8.17 (d, J = 10.2 Hz, 1H), 7.38 (s, 1H), 6.56 (s, 1H), 5.74 (d, J = 19.4 Hz, 1H), 5.52 – 5.40 (m, 3H), 5.16 (s, 1H), 3.44 (dd, J = 16.3, 4.1 Hz, 1H), 3.19 (t, J = 13.9 Hz, 1H), 2.57 (d, J = 14.0 Hz, 1H), 2.26 (t, J = 14.3 Hz, 1H), 1.89 (hept, J = 7.0 Hz, 2H), 0.89 (t, J = 7.3 Hz, 3H). MS m/z 456.0 (M+H). HPLC rt = 1.395 min (Agilent 1200; Chromatographic column: Waters XBridge C18 4.6*50mm, 3.5μm; Flow: 2.0mL/min; Gradient elute: 5.0%-95.0%-95.0%-5.0%-5.0%, 0.00min-1.50min-2.50min-3.00min; Temperature : 40°C; Phase: A: Acetonitrile, B: H₂O (0.05% TFA); Wavelength: 214 nm/254 nm).

1.2 Preparation of linker-payload intermediate 1 – Scheme B

Scheme B: Synthetic Route to linker-payload intermediate 1



[00120] Linker-payload intermediate 1 can be synthesized by a conventional solid phase polypeptide synthesis using Rink-amide-MBHA-resin. Fmoc was used to protect the amino acid in the linking unit. The coupling reagent was selected from HOBT, HOAt/DIC, DCC, EDCI or HATU. After synthesis, the product was cleaved from resin using TFA/TIS/H₂O solution. The product was purified by prep-HPLC, lyophilized and stored for use. MS m/z: [M-H]⁻ = 1382.6.

1.3 Preparation of Linker-Payload – Scheme C

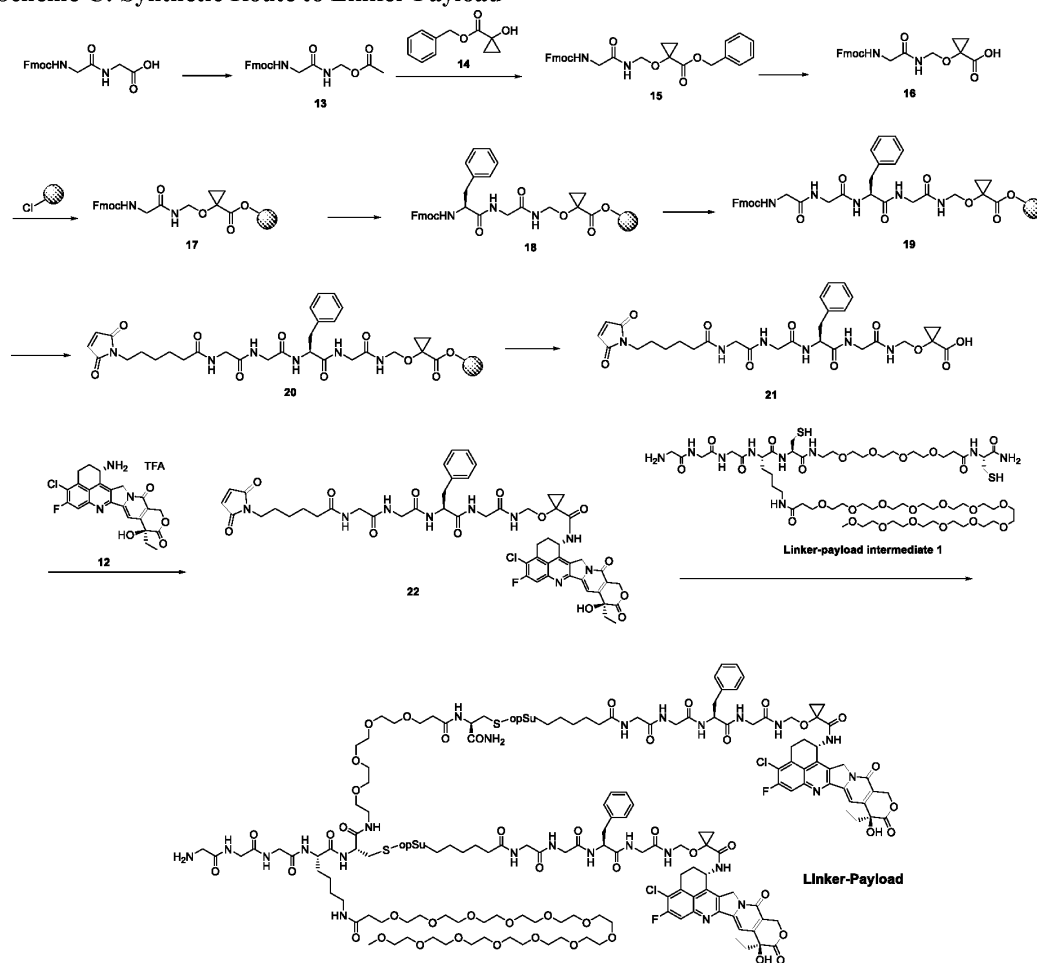
1.3.1 Preparation of Compound 13

[00121] 4.33 g Fmoc-Gly-Gly-OH and 6.84 g $\text{Pb}(\text{OAc})_4$ were weighed and added into a 500 ml single-neck round bottom flask. Anhydrous THF/Toluene (120/40 ml) was added under nitrogen atmosphere and stirred for dissolving. Then 1.16 mL of pyridine was added to the reaction system. The reaction system was heated to 80°C and refluxed for 5hr under nitrogen atmosphere. Samples were taken and detected by HPLC to monitor the reaction. The reaction system was cooled to room temperature, filtered, and the filter cake was washed with EA for 3 times. The filtrates were combined and concentrated to dryness. Column chromatography was performed (PE: EA = 100: 0 - 50: 100) to give about 2000 mg of the Compound 13 as a white solid with a yield of 44%.

1.3.2 Preparation of Compound 15

[00122] Compound 13 (200 mg) was weighed into a 100 ml single-neck round bottom flask. Then 15 ml THF was added and stirred for dissolving. Then Compound 14 (312mg, 3.0 e.q.) and TsOH·H₂O (15 mg, 0.15 e.q.) were added to the reaction system. The reaction system was reacted overnight at room temperature. Samples were taken and detected by TLC (PE/EA=1: 1) to monitor the reaction. Saturated sodium bicarbonate solution was added to quench reaction. Extraction was conducted with EA for 3 times. The organic phase was combined and washed with saline, dried with anhydrous magnesium sulfate and concentrated. The crude product was purified by column chromatography (PE: EA = 5: 1 - 1: 1) to give about 80 mg of Compound 15 as a colorless oil with a yield of 29%. MS m/z: [M+H]⁺ = 501.1

Scheme C: Synthetic Route to Linker Payload



1.3.3 Preparation of Compound 16

[00123] Compound 15 (200 mg) was weighed into a 100 ml single-neck round bottom flask. Then 10 ml of EtOH and 5 ml of EA were added with complete dissolution. Then 40 mg of palladium carbon was added to the reaction system under nitrogen atmosphere, and the reaction system was purged with hydrogen gas for three times. The reaction system was kept under hydrogen atmosphere and stirred for 0.5 hour at room temperature. Samples were taken and detected by TLC (DCM/MeOH=10: 1) to monitor the reaction. The reaction system was filtered, and the filter cake was washed with EA for 3 times. The filtrates were combined and concentrated to dryness to give 200 mg product in white solid with 100% yield. The product can be directly used in the next reaction without purification. MS m/z: [M-H]⁻ = 409.4.

1.3.4 Preparation of Compound 21 via solid-phase synthesis

1.3.4.1 Step A – Formation of Compound 17

[00124] 2.0 g of dichlororesin was weighed and placed in a polypeptide synthesis tube. DCM (10 ml) was added and swelled at room temperature for 30 minutes. The solvent was removed by vacuum suction. The resin was washed twice with DCM, with a volume of 7 mL and a time length of 1 minute for each wash. The solvent was removed by vacuum suction. Then Compound 16 (200 mg) was weighed and added into a 50 ml centrifuge tube. DCM (about 10 ml) was added. the solid was dissolved by shaking. Added to the above resin. Stirring was conducted to soak all the resin in the solution (if there was resin attached to the tube wall, a small amount of DCM was used to wash the tube wall). Stirring was conducted for 4-5 hours. After the reaction was complete methanol was added. Stirring was conducted for 30 min. The solvent was removed by vacuum suction. The resin was washed with DMF once, methanol once, DMF once, methanol once and DMF twice in sequence, with a volume of 10 mL and a

time length of 1 minute for each wash. The solvent was removed by vacuum suction. A small amount of dry resin was taken for ninhydrin detection. The resin was colorless and transparent, and the solution was yellowish, indicating it qualified for the next coupling step.

1.3.4.2 Step B – Formation of Compound 18

[00125] The deprotection of Compound 17 was conducted twice by adding 10 mL of 20% piperidine/DMF solution and reacting for 10 minutes for each time. After the reaction was complete, the solution was removed by vacuum suction. The resin was washed with DMF twice, methanol once, DMF once, methanol once and DMF twice in sequence, with a volume of 10 mL and a time length of 1 minute for each wash. The solvent was removed by vacuum suction. A small amount of dry resin was taken for ninhydrin detection. Both the resin and solution were dark blue.

[00126] To a 50 mL centrifuge tube was added 563 mg Fmoc-Phe-OH and 197 mg HOBt. Then about 7 mL DMF was added. The solid was dissolved by shaking. Then 0.24 mL DIC was added. Activated for 10-30 minutes to give the activated reaction solution. 3 molar equivalent of activated reaction solution was added to the resin. Stirring was conducted to soak the resin completely in the solution. Stirring was conducted for 2-3 hours. After the reaction was complete, the solvent was removed by vacuum suction. The resin was washed with DMF twice, methanol once, DMF once, methanol once and DMF twice in sequence, with a volume of 10 mL and a time length of 1 minute for each wash. The solvent was removed by vacuum suction. A small amount of dry resin was taken for ninhydrin detection. The resin was colorless and transparent, and the solution was yellowish, indicating it qualified for the next coupling step.

1.3.4.3 Step C – Formation of Compound 19

[00127] The deprotection of Compound 18 was conducted twice by adding 10 mL of 20% piperidine/DMF solution and reacting for 10 minutes for each time. After the reaction was complete, the solution was removed by vacuum suction. The resin was washed with DMF twice, methanol once, DMF once, methanol once and DMF twice in sequence, with a volume of 10 mL and a time length of 1 minute for each wash. The solvent was removed by vacuum suction. A small amount of dry resin was taken for ninhydrin detection. Both the resin and solution were dark blue.

[00128] To a 50 mL centrifuge tube was added 531 mg Fmoc-Gly-Gly-OH and 197mg HOBt. Then about 10 mL DMF was added. The solid was dissolved by shaking. Then 0.24 mL DIC was added. Activated for 10-30 minutes to give the activated reaction solution. 3 molar equivalent of activated reaction solution was added to the resin. Stirring was conducted to soak the resin completely in the solution. Stirring was conducted for 2-3 hours. After the reaction was complete, the reaction solution was removed by vacuum suction. The resin was washed with DMF twice, methanol once, DMF once, methanol once and DMF twice in sequence, with a volume of 10 mL and a time length of 1 minute for each wash. The solvent was removed by vacuum suction. A small amount of dry resin was taken for ninhydrin detection. The resin was colorless and transparent, and the solution was yellowish, indicating it qualified for the next coupling step.

1.3.4.4 Step D – Formation of Compound 20

[00129] The deprotection of Compound 19 was conducted twice by adding 10 mL 20% piperidine/DMF solution and reacting for 10 minutes for each time. After the reaction was complete, the solution was removed by vacuum suction. The resin was washed with DMF twice, methanol once, DMF once, methanol once and DMF twice in sequence, with a volume of 10 mL and a time length of 1 minute for each wash. The solvent was removed by vacuum suction. A small amount of dry resin was taken for ninhydrin detection. Both the resin and solution were dark blue. Then 462 mg MC-OSu was placed in a 50 mL centrifuge tube, and 10 mL DMF was added. The solid was dissolved by shaking. Then 0.24 mL DIEA was added to the resin. Stirring was conducted to soak the resin completely in the solution. Stirring was conducted for 2-3 hours. After the reaction was complete, the reaction solution was removed by vacuum suction. The resin was washed with DMF twice, methanol once, DMF once, methanol once and DMF twice in sequence, with a volume of 10 mL and a time length of 1 minute for each wash. The solvent was removed by vacuum suction. A small amount of dry resin was taken for ninhydrin detection. The resin was colorless and transparent, and the solution was yellowish, indicating it qualified for the next coupling step.

1.3.4.5 Step E – Formation of Compound 21

[00130] The Compound 20 resin was washed twice with 10 mL of methanol. Then the solvent was removed thoroughly by vacuum suction. The resin was poured out and weighed. The lysis buffer was

prepared in a 250 mL conical flask, wherein the ratio of TFE/DCM was 80%/20%, and the volume was 7-8 times relative to the weight of peptide resin. The lysis buffer was added to the peptide resin and shaken well. The resin was fully soaked in the lysis buffer, and lysis was carried out at room temperature for 2-3 hours. The lysis buffer was then filtered out using a simple filter made of a syringe, and the resin was washed with 1-2 ml DCM and discarded. Then 150 mL precooled anhydrous ether was added to the lysis buffer, shaken well and then stood for 20-30 minutes. Using a 50 mL centrifuge tube, the above system was centrifuged in a centrifuge at 3500 rpm for 3 minutes, and the supernatant was poured out and discarded. The solid was shaken with precooled anhydrous ether, washed once under ultrasound, centrifuged at 3500rpm for 3 minutes, and the supernatant was poured out and discarded. The solid was placed in a centrifuge tube and allowed to air dry overnight, and then subjected to preparative purification to give 125 mg of Compound 21 as a white solid with a yield of 40%. MS m/z: $[M-H]^- = 641.5$.

1.3.5 Preparation of Compound 22

[00131] 150 mg of Compound 21 and 55 mg of TSTU were weighed and added into a 10 mL single-neck round bottom flask, and anhydrous DMF (3 mL) was added under nitrogen atmosphere and stirred for 20 min. Then 18 mg of Compound 12 and 20 μ l DIEA were added in sequence to the reaction system. Stirring was conducted at room temperature for 2-8 hours under nitrogen atmosphere. Samples were taken and detected by HPLC to monitor the reaction, and once completed, the reaction system was subjected to preparative purification, and the product was collected and lyophilized to give about 22mg of Compound 22 as a yellowish solid. MS m/z: $[M+H]^+ = 1081.0$.

1.3.6 Preparation of Linker-Payload

[00132] Compound 22 (30 mg) was weighed and added into a 10 ml single-neck round bottom flask, and purified water (2 ml) was added. After stirring to dissolve, DMF solution (2 ml) containing Linker-payload intermediate 1 (19.5 mg) was added to the reaction system and stirred. After reacting overnight, HPLC was used to monitor the reaction until all of the raw material had converted into intermediates. The reaction mixture was directly added with an appropriate amount of Tris Base solution or other solution that promotes the ring-opening reaction, and the reaction was performed at 0-40°C for another 0.2-20h. The reaction was monitored by HPLC until all the intermediates were consumed and then quenched by acetic acid solution.

[00133] The reaction mixture was subjected to preparative purification, and the target product was collected and lyophilized to give about 25mg of Linker-Payload as a yellowish solid. MS m/z: $[(M+3H)/3]^+ = 1194.4$.

1.4 Preparation of antibody GQhRS7

1.4.1 Construction of expression vectors encoding antibody

[00134] The sequence of GQhRS7 is based on the amino acid sequence of hRS7, which is a known antibody (WO03074566A2), but GALPETGG has been introduced at the C-terminus of each light chain, wherein LPETGG is the recognition sequence of the ligase donor substrate, and GA is a spacer sequence.

[00135] The antibody GQhRS7 comprises a heavy chain sequence (SEQ ID NO: 1) and a light chain sequence (SEQ ID NO: 2) as follows:

SEQ ID NO: 1:

QVQLQQSGSELKKPGASVKVSCASGYTFTNYGMNWVKQAPGQGLKWMGWINTYTGEPT
YTDDFKGRFAFSLDTSVSTAYLQISSLKADDTAVYFCARGGFGSSYWFYFDVWGQGLSLTVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVTVPPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMH
EALHNHYTQKSLSLSPGK.

SEQ ID NO: 2:

DIQLTQSPSSLSASVGDRVSITCKASQDVSI A VAWYQQKPGKAPKLLIYSASYRYTGVPDRFS
GSGSGTDFLTITSLQPEDFAVYYCQQHYITPLTFGAGTKVEIKRTVAAPSVFIFPPSDEQLKSG
TASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGECGALPETGG

[00136] To generate expression vectors encoding the light chain the nucleic acid sequence (SEQ ID NO: 2) was cloned into a pCDNA 3.3 vector (Life technology); to generate the expression vectors

encoding the heavy chain the nucleic acid sequence (SEQ ID NO: 1) was cloned into a pCDNA 3.3 vector (Life technology).

1.4.2 Expression of antibody

[00137] Plasmids encoding the light and heavy chains of GQhRS7 were paired and mixed at a mass ratio of 2:1. The plasmid pair and the PEIMAX (Polyscience) transfection reagent were separately diluted in HEK293F basic medium and then mixed evenly. Transfection was performed by electroporation using Neon[®] Transfection system. The mixture was let stand at room temperature and added to the HEK293F seed cell culture. The cell was cultured at 32 for 24 h and sampled for cell density and viability analysis and supplemented with 10% volume of HEK293F feed medium. Then the culture temperature was shifted to 32°C for the following culture. At 72 h of incubation, the cell culture was sampled again for cell density and viability analysis. At 144 h of incubation, the cell culture was sampled for cell density and viability analysis.

1.4.3 Purification of antibody

[00138] GQhRS7 was purified by affinity chromatography following the manufacturer's instruction. Briefly, the chromatography column (BestChrom, Shanghai, China) was packed with the MabSelect SureLX resin (GE Healthcare) and equilibrated with 50 mM Tris, 150 mM NaCl, pH 7.4. Then the supernatant of the cell culture was obtained and applied onto the column. The column was washed with 50 mM Tris, 150 mM NaCl, pH 7.4 to remove non-specifically bound proteins. Then the antibody was eluted by 50 mM citrate Buffer, pH 3.5 and the antibody-containing eluate was adjusted to pH 6.5 using 1 M Tris-HCl, pH 9.0. Finally, the buffer of the antibody was exchanged to 50 mM Tris, 150 mM NaCl, pH 7.4 by an Anicon Ultra-15 centrifugal Filter (Merk Millipore).

1.5 Preparation of ADC

[00139] The ADC is prepared by the ligase-catalysed site-specific conjugation of the GQhRS7 antibody and the linker-payload compound (suitable conjugations methods can be found in WO2015165413A1).

[00140] The antibody GQhRS7 was treated by ultrafiltration, dialysis or desalting column. The storage solution was replaced with a ligase buffer. ADC was prepared by coupling reaction of GQhRS7 with the Linker-Payload (described in Example 1.3 above), under the catalysis of a wild type Sortase A or a mutant ligase optimized and engineered based thereon. In the ligase buffer, the modified antibody and linker-payload were thoroughly mixed at a molar ratio of 1:1 to 1:100, and added to a solid phase coupling system. The solid phase coupling system comprised a ligase immobilized on the matrix of the solid phase coupling system. The immobilized ligase catalyzed the coupling reaction of the antibody GQhRS7 with Linker-Payload. The coupling reaction was carried out at 4 – 40°C for 0.5 – 20 h. After the reaction was completed, the reaction mixture was subjected to ultrafiltration or dialysis to remove unreacted intermediate, giving ADC. The ADC was stored at 4°C or -80°C in a buffer containing 20 mM citric acid, 200 mM NaCl, pH 5.0.

1.6 Analysis & Characterisation of ADC

HIC-HPLC detection and analysis of ADC

[00141] The DAR (drug-to-antibody ratio) distribution of the ADC was analysed by HIC-HPLC. The coupled product was found to mainly contain ADC with DAR of 3.4-3.5.

Example 2: Biological Activity of ADC

2.1 Human Trop 2 Binding Affinity

[00142] Human Trop 2 ECD at concentration of 0.5 µg/mL was coated on 96-well plates and incubated at 4°C overnight. The plates were then blocked with 3% BSA-PBST for 1 h at room temperature. After washing with PBST (0.05% Tween), ADC, Trodelvy[®] (comparative Trop 2 ADC) or antibody (GQhRS7) at different concentrations were added onto the 96-well plates, and then incubated at room temperature for 60 min. After incubation goat anti-human FC secondary antibody (HRP) (Sinobiological, SSA001) was added at a ratio of 1:100000 and incubated at room temperature for 60 min. Following the wash, the plate was treated with TMB solution (Sigma, T0440) as an HRP substrate, and the reaction was stopped with 1 M H₂SO₄. The absorbance for each well was detected at 450 nm wavelength.

[00143] Figure 1 shows the Trop 2 binding affinity curves for ADC, Trodelvy[®] and GQhRS7. The results demonstrate that ADC has similar Trop 2 binding affinity to GQhRS7 and Trodelvy[®].

2.2 Effect on Tumour Cell Proliferation

[00144] Trop 2 positive cancer cells BxPC-3, FaDu, and NCI-N87 (3000 to 5000 cells) were plated in 96-well plates, and cells were able to attach overnight. Cells were treated with ADC, DS1062a

(comparative Trop 2 ADC – as described in US 2016/0297890A), or antibody (GQhRS7) at various concentrations for 168 h. Cell viabilities were examined by CellTiter-Glo® Luminescent Cell Viability Assay, and percentage of cell viability was calculated.

[00145] Figures 2 to 4 show the cell viability curves for ADC, DS1062a, and GQhRS7 on BxPC-3, FaDu and NCI-N87 cells respectively. In Trop2 positive BxPC-3, FaDu and NCI-N87 cells, ADC exhibited IC₅₀ values of 0.226 nM, 0.128 nM, and 0.742 nM respectively, while DS1062a exhibited IC₅₀ values of 0.692 nM, 0.170 nM, and 2.101 nM respectively. Antibody alone (GQhRS7) did not show any significant effect on cell viability at the concentrations tested.

2.3 Bystander Killing Effect of ADC against HepG2 in BxPC-3/HepG2 co-culture assay

[00146] The cell concentration of Trop2-positive BxPC-3 cells and Trop2-negative HepG2 cells were adjusted to 1×10^6 cells/mL, and 300 μ L per well (cell volume BxPC-3 : HepG2 = 2 : 1) was inoculated into six-well plates, and 2.7 mL of 45% RPMI-1640 + 45% DMEM + 10% FBS medium was supplemented. The cells were incubated overnight in a cell incubator at 37°C, 5% CO₂. 3 mL of 20 nM ADC and DS1062a were added to cells cultured overnight (final drug concentration of 10 nM per well) respectively. A negative control group was set: 3 mL of 45% RPMI-1640 + 45% DMEM + 10% FBS medium was added to each well. After the treatment, the cells were moved to the incubator and incubated for 96 h. After the incubation, cells were digested, and washed once with 1X PBS, then transferred to a flow tube, and centrifuged at 2000 rpm for 3 min. The supernatant was then discarded, and the cell amount and cell viability were detected. A certain amount of cells were washed with 1X PBS, the supernatant was discarded after centrifugation, 200 μ L of 100 nM anti-human Trop2 antibody was added, and the cells were mixed and incubated at 4°C for 30 min. The cells were washed with 1X PBS, the supernatant was discarded after centrifugation, 200 μ L of 5 μ g/mL human IgG Fc antibody was added, and the resulting cells were continued to incubate at 4°C for 30 min after mixing. Finally, the cells were washed with 1X PBS, the supernatant was discarded after centrifugation, and the cells were resuspended in PBS, detected by flow cytometry and analyzed by FlowJo software. The results are as shown in Table A and demonstrate that the bystander killing effect of ADC was better than that of the comparative ADC - DS1062a.

Table A: Bystander killing test results of ADC and DS1062a

Groups	Number of viable cells (10 ⁴)	Cell proportion (%)		Amount of cells (10 ⁴)	
		BxPC-3	HepG2	BxPC-3	HepG2
Control	452.0	22.4	77.6	101.25	350.75
DS1062a	157.0	7.8	92.2	12.25	144.75
ADC	70.0	21.8	78.2	15.26	54.74

2.4 In vivo mouse xenograft tumor growth inhibition

[00147] 0.2 mL of cancer cells (human pancreatic cancer cell line BxPC-3; pharyngeal squamous cell carcinoma line FaDu; or gastric cancer cell line NCI-N87) as a suspension (cell density = 10×10^6 cells/mL in Matrigel buffer (PBS : Matrigel = 1:1)) was subcutaneously injected into the right scapula of SPF female BALB/c nude mice aged 6-8 weeks. The tumor diameter was measured with a vernier caliper and the tumor volume was calculated according to the formula $V = 0.5 a \times b^2$ (where a is the longest diameter of the tumor and b is the shortest diameter of the tumor).

[00148] For BxPC-3 tumors, 6 days after cell inoculation, when the average tumor volume was about 151 mm³, animals were randomly divided into vehicle control group, DS1062a 3 mg/kg group and ADC 3 mg/kg group, with 6 animals in each group.

[00149] For FaDu tumors, 11 days after cell inoculation, when the average tumor volume was about 123 mm³, animals were randomly divided into vehicle control group, DS1062a 3 mg/kg group, and ADC 3 mg/kg group, with 6 animals in each group.

[00150] For NCI-N87 tumors, 8 days after cell inoculation, when the average tumor volume was about 188 mm³, animals were randomly divided into vehicle control group, DS1062a 3 mg/kg group, and ADC 3 mg/kg group, with 6 animals in each group.

[00151] Animals in each group were administered by tail vein injection, and the control group was given an equal volume of vehicle. The tumor volume of animals in each group was measured twice a week within 35 days after administration, and the tumor volume of animals on day 35 was compared between groups. T/C and TGI values were calculated using tumor volume. The calculation formula is as follows: $T/C\% = T_{RTV} / C_{RTV} \times 100\%$ (T_{RTV} : RTV of the treatment group; C_{RTV} : RTV of the vehicle control group). The relative tumor volume (RTV) was calculated based on the results of tumor

measurement, and the calculation formula was $RTV = V_t / V_0$, where V_0 was the average tumor volume measured at the time of grouping (i.e., D0), V_t was the average tumor volume at one measurement, and T_{RTV} and C_{RTV} took the same day of data. Calculation of TGI (%): $TGI (\%) = [1 - (\text{average tumor volume at the end of administration of a treatment group} - \text{average tumor volume at the beginning of administration of the treatment group}) / (\text{average tumor volume at the end of treatment of the vehicle control group} - \text{the average tumor volume at the beginning of treatment of the vehicle control group})] \times 100\%$. The results are shown in Table B1. In all three tumor types, ADC inhibited tumor growth and to a greater degree than observed with the comparative ADC – DS1062a. In the FaDu tumor cohort all six mice had complete tumor regression after treatment with ADC.

Table B1: Inhibitory effect of ADC on mouse xenograft tumors

Administration group	Cell line	Tumor volume (mm ³)	Tumor volume (mm ³)	T/C	TGI	p value
		Day 0	Day 28	(%)	(%)	
Vehicle	BxPC-3	151 ± 13	906 ± 82	--	--	--
ADC		151 ± 12	116 ± 21	12.72	104.54	0.001
DS1062a		151 ± 12	540 ± 74	58.49	48.52	0.049
Vehicle	FaDu	123 ± 12	1,676 ± 211	--	--	--
ADC		123 ± 12	0 ± 0	0.00	107.90	0.003
DS1062a		123 ± 13	28 ± 14	1.68	106.11	0.003
Vehicle	NCI-N87	189 ± 8	634 ± 84	--	--	--
ADC		188 ± 11	89 ± 21	14.08	122.25	<0.001
DS1062a		188 ± 11	255 ± 35	40.30	84.96	<0.001

2.5 *In vivo* efficacy evaluation of ADC on MDA-MB-468

[00152] The MDA-MB-468 (Trop-2 positive human breast cancer cells; ATCC, HTB-132) cells were maintained *in vitro* as a monolayer culture in L-15 medium supplemented with 10% fetal bovine serum and 1% Antibiotic-Antimycotic at 37 °C in an atmosphere of 0% CO₂ in air. The cells growing in an exponential growth phase were harvested and counted for tumor inoculation. 10x10⁶ MDA-MB-468 cells in 0.2 mL of PBS with Matrigel (1:1) were inoculated subcutaneously in the right flank in BALB/c Nude mice. After 24 days, when tumor volume reached 187 mm³ on average, the tumor bearing mice were assigned and administrated intravenously of ADC at 0.5 mg/kg, 1.5 mg/kg and 4.5 mg/kg, Trodelvy[®] at 4.5 mg/kg, or DS1062a at 4.5 mg/kg. The tumor volume was measured twice weekly with a caliper. T/C and TGI values were calculated using tumor volume as described in Example 2.4.

[00153] The results are shown in Table B2 and Figure 5. ADC showed significantly better efficacy than Trodelvy[®] and slightly better efficacy than DS1062a at the equivalent dose.

Table B2: Inhibitory effect of ADC on MDA-MB-468 mouse xenograft tumors

Treatment	N	Tumor volume (mm ³) on day 35	T/C (%)	TGI (%)	p value
Vehicle	6	678 ± 68	--	--	--
Trodelvy [®] , 4.5 mg/kg	6	535 ± 60	78.73	29.24	0.701
DS1062a, 4.5 mg/kg	6	9 ± 9	1.35	136.06	0.001
ADC, 0.5 mg/kg	5	588 ± 85	86.04	18.65	0.975
ADC, 1.5 mg/kg	6	202 ± 41	29.77	96.85	0.004
ADC, 4.5 mg/kg	6	0 ± 0	0.00	137.93	0.002

Example 3: Initial Formulation Screening

3

3.1 Formation of ADC buffer formulations 1-12

[00154] 2 L of the 12 buffer solutions according to Table C were prepared. A sample of ADC in citrate buffer (50.1 mg/ml) was transferred into an ultrafiltration centrifuge tube (x 12) and then buffer solutions according to Table C were added and ultrafiltration centrifugation was carried out to change the liquid. Each centrifugation was carried out at 3500 rpm for 15min, so that the fluid replacement volume ratio was not less than 8 times, and the fluid replacement rate was not less than 99%. After the medium replacement was completed, the protein concentration, volume, and pH value were measured and the samples were diluted with the corresponding buffer to a target concentration of 20.0 mg/ml.

[00155] The formulations 1-12 were aseptically filled into 2R vials (1.0 ml/bottle) on a clean bench and plugged and capped.

[00156] Formulations 1-12 were then stored under various conditions (-20 °C, 2-8 °C, 25 °C, and 40 °C) for up to 4 weeks and analysed periodically as described in section 3.2.

Table C: Summary of initial formulations

Formulation	Buffer	pH	Concentration of protein in ADC
Formulation 1	20 mM Acetate buffer	4.8	20 mg/ml
Formulation 2	20 mM Acetate buffer	5.2	
Formulation 3	20 mM Acetate buffer	5.4	
Formulation 4	20 mM Citrate buffer	5.2	
Formulation 5	20 mM Citrate buffer	5.6	
Formulation 6	20 mM Citrate buffer	6.0	
Formulation 7	20 mM Histidine buffer	5.4	
Formulation 8	20 mM Histidine buffer	5.8	
Formulation 9	20 mM Histidine buffer	6.2	
Formulation 10	20 mM Succinate buffer	5.6	
Formulation 11	20 mM Succinate buffer	6.0	
Formulation 12	20 mM Succinate buffer	6.4	

3.2 Analysis of ADC buffer formulations 1-12

[00157] The formulations were analysed according to the techniques described in the General Methods section above.

3.3 Results of Initial Formulation Screening

[00158] The protein concentration and pH of the buffer formulations after 2-4 weeks storage under the various conditions is shown in Table D. No significant changes in either protein concentration or pH were observed under these conditions.

Table D: Protein concentrations and pH of formulations 1-12 after up to 4 weeks storage

	Protein Concentration (mg/ml)								pH							
	T=0	2-8°C		20°C		25°C		40°C		T=0	2-8°C		25°C		40°C	
		4W	4W	2W	4W	2W	4W	4W	4W		4W	4W	4W	4W		
Formulation 1	20.6	20.4	20.3	20.4	20.5	20.5	20.4	4.80	4.81	4.83	4.81	4.80				
Formulation 2	20.5	20.3	20.4	20.5	20.7	20.5	20.7	5.20	5.17	5.17	5.17	5.19				
Formulation 3	20.6	20.6	20.6	20.6	20.7	20.6	20.5	5.40	5.38	5.38	5.38	5.39				
Formulation 4	20.2	20.1	19.8	20.2	20.0	20.1	19.9	5.21	5.23	5.21	5.23	5.22				
Formulation 5	20.7	20.8	20.0	20.7	20.7	20.7	19.9	5.61	5.61	5.61	5.61	5.60				
Formulation 6	20.6	20.4	20.5	20.6	20.5	20.6	20.4	6.00	5.99	5.99	6.00	6.01				
Formulation 7	20.5	20.4	20.4	20.5	20.6	20.5	20.6	5.40	5.40	5.40	5.39	5.35				
Formulation 8	20.6	20.3	20.6	20.7	20.4	20.7	20.6	5.80	5.77	5.78	5.77	5.75				
Formulation 9	20.4	20.5	20.3	20.3	20.4	20.4	20.5	6.20	6.18	6.19	6.19	6.19				
Formulation 10	20.4	20.2	20.3	20.4	20.4	20.5	20.3	5.60	5.61	5.62	5.62	5.63				
Formulation 11	20.5	20.4	20.3	20.4	20.2	20.5	20.2	6.01	6.00	6.00	6.00	5.99				
Formulation 12	19.2	19.1	19.2	19.1	19.3	19.2	19.3	6.38	6.37	6.37	6.37	6.37				

[00159] In terms of appearance of the formulations, no obvious foreign matter was observed in any of the samples at T=0. Except for Formulation 1, which was a colourless and clear liquid, the other formulations were all colourless and slightly opalescent liquids. Formulations 4, 5 and 6 produced fine, visible particles after being stored at 40 °C for 4 weeks, indicating that the ADC is more likely to produce particles in the citrate buffer system. In addition, Formulations 1 to 6 were taken out and thawed at -20 °C and left to stand for 4 hours. After visual inspection, a large number of small bubbles appeared, which did not disappear after four hours of standing.

[00160] The evaluation of the insoluble particle levels of Formulations 1-12 during the stability storage process under different conditions (Tables E1 and E2) shows that the number of insoluble particles is relatively higher in the citrate buffer (Formulations 4-6) and succinate buffer (Formulations 10-12) systems, compared to the acetate buffer (Formulations 1-3) and histidine buffer (Formulations 7-9) systems.

Table E1: Insoluble particle levels at different temperatures and times (A: $\geq 2\mu\text{m}$; B: $\geq 5\mu\text{m}$; unit: number/ml)

	T=0		2-8°C				25°C				40°C				30°C			
			2W		4W		2W		4W		1W		4W		2W		4W	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Formulation 1	395	150	527	234	135	39	354	77	545	177	859	224	1374	277	3185	1185	719	215
Formulation 2	479	179	2705	1057	142	45	647	120	1014	174	925	220	1769	480	5427	2892	1105	330
Formulation 3	397	112	280	79	187	92	245	44	760	214	1112	245	2129	419	8714	4517	1165	349
Formulation 4	1967	495	807	199	3672	345	8204	1607	6665	939	11904	2295	7264	1640	9897	3605	10619	5157
Formulation 5	4745	2009	1539	195	3809	474	8421	2979	9884	1532	5439	1622	7915	1594	6782	1430	5254	937
Formulation 6	3612	770	1625	207	1304	164	6777	799	13135	4485	12957	3857	7047	1224	7017	1940	5272	907
Formulation 7	465	134	440	165	205	92	560	127	1159	269	1205	269	1615	252	5740	1669	809	285
Formulation 8	365	127	640	145	247	114	1749	425	764	129	2197	417	2370	372	3749	2250	1979	410
Formulation 9	1105	325	250	85	245	99	2002	329	1285	314	2457	637	2582	465	3085	1064	6154	1900
Formulation 10	2699	477	1185	229	834	109	3832	375	4744	519	6705	1164	6335	1337	2617	705	2917	887
Formulation 11	282	132	1434	160	819	120	6740	1629	6212	1492	1230	479	6064	1160	4817	1902	4502	849
Formulation 12	2894	617	3912	6793	4355	555	5387	442	7905	944	7979	1612	5544	1697	7504	2389	3339	917

Table E2: Insoluble particle levels at different temperatures and times (C: $\geq 10\mu\text{m}$; D: $\geq 25\mu\text{m}$; unit: number/ml)

	T=0		2-8°C				25°C				40°C				30°C			
			2W		4W		2W		4W		1W		4W		2W		4W	
	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D
Formulation 1	55	10	74	2	12	0	22	9	60	0	45	0	64	5	405	14	52	2
Formulation 2	70	9	260	4	9	2	32	0	30	0	55	4	108	2	925	14	52	0
Formulation 3	39	4	22	0	35	0	20	2	52	2	69	2	79	5	1077	4	54	2
Formulation 4	117	19	55	2	94	0	319	7	84	5	439	7	207	2	1220	10	1366	12
Formulation 5	465	2	27	4	74	2	900	47	139	2	364	4	189	0	330	15	199	2
Formulation 6	132	0	39	0	44	0	97	2	689	9	617	9	85	2	362	9	150	4
Formulation 7	27	4	40	2	34	0	34	4	52	5	100	17	52	2	204	9	90	5
Formulation 8	47	2	39	2	52	0	104	5	42	2	112	5	72	4	355	30	100	4
Formulation 9	87	37	34	0	47	5	59	5	87	2	197	32	122	17	289	2	409	0
Formulation 10	97	9	37	0	39	2	82	2	64	2	205	4	199	5	152	2	269	12
Formulation 11	39	2	17	0	39	2	574	19	364	22	135	5	179	2	205	5	305	64
Formulation 12	110	4	165	4	112	2	39	2	80	4	232	0	349	12	352	0	200	0

[00161] According to the SEC-HPLC aggregate content results in Table F1, under high temperature and accelerated conditions, the amount of aggregates increased in all formulations, however, there was a greater increase for formulations 4-6 and 10-12, indicating that the ADC aggregates are more likely to form in citrate and succinate buffers. After being placed at 2-8°C and -20°C for 4 weeks, no significant increase in aggregates occurred in any formulation. In addition, it can be found that for a given buffer system, the higher the pH value, the faster the rate of aggregate formation.

Table F1: Percentage aggregate contents (SEC-HPLC) of Formulations 1-12 at different temperatures and times

	T=0	2-8°C		20°C		25°C			40°C		
		2W	4W	2W	4W	1W	2W	4W	1W	2W	4W
Formulation 1	2.9	2.8	2.7	2.9	2.8	2.9	2.7	2.7	2.7	3.0	3.7
Formulation 2	3.0	2.6	2.9	2.7	2.9	2.8	2.9	3.0	2.9	3.3	4.4
Formulation 3	3.0	3.0	2.9	3.1	3.0	2.7	3.0	3.1	2.9	3.6	4.8
Formulation 4	2.9	3.9	2.9	3.1	2.9	3.5	3.1	3.1	3.3	4.0	5.9
Formulation 5	2.6	2.9	2.6	2.8	2.6	2.9	2.8	3.0	2.9	3.7	5.6
Formulation 6	2.8	2.7	2.9	2.9	2.8	3.0	3.2	3.3	3.1	4.2	6.2
Formulation 7	2.9	3.2	2.8	2.9	2.8	2.9	2.8	2.9	2.8	3.3	4.4
Formulation 8	2.9	3.3	2.9	3.0	2.8	3.0	2.9	3.0	4.3	3.4	4.7
Formulation 9	2.9	3.0	3.0	3.0	2.9	2.7	3.3	3.2	3.3	3.6	5.3
Formulation 10	3.2	2.9	3.2	3.3	3.1	3.2	3.0	3.5	3.7	4.0	5.6
Formulation 11	3.3	3.0	3.3	3.2	3.2	3.0	3.3	4.4	3.1	4.4	6.2
Formulation 12	3.8	2.9	3.9	4.0	3.8	4.0	3.9	3.7	3.4	5.2	7.1

Table F2: Percentage monomer contents (SEC-HPLC) of Formulations 1-12 at different temperatures and times

	T=0	2-8 °C		-20 °C		25 °C			40 °C		
		2W	4W	2W	4W	1W	2W	4W	1W	2W	4W
Formulation 1	97.0	97.1	97.2	96.9	97.2	96.8	97.1	97.2	97.0	96.6	95.8
Formulation 2	96.8	97.4	97.1	97.3	97.1	97.0	96.9	96.9	96.9	96.4	95.2
Formulation 3	96.9	96.8	97.0	96.9	97.1	97.1	96.8	96.8	96.9	96.1	94.9
Formulation 4	97.1	95.9	97.0	96.9	97.1	96.4	96.7	96.7	96.4	95.7	93.4
Formulation 5	97.3	97.1	97.4	97.0	97.4	96.9	97.0	96.9	96.9	95.9	93.9
Formulation 6	97.1	97.3	97.1	96.9	97.2	96.8	96.7	96.6	96.7	95.5	93.3
Formulation 7	97.0	96.8	97.2	96.9	97.2	97.0	97.0	97.0	96.9	96.4	95.1
Formulation 8	97.0	96.5	97.1	96.9	97.2	96.8	96.9	96.9	95.6	96.3	94.9
Formulation 9	96.9	96.9	97.0	96.8	97.1	97.1	96.7	96.7	96.5	96.0	94.2
Formulation 10	96.6	97.0	96.8	96.6	96.9	96.6	96.8	96.4	96.1	95.7	94.0
Formulation 11	96.5	96.9	96.7	96.8	96.8	96.8	96.5	95.5	96.7	95.5	93.4
Formulation 12	96.0	97.0	96.1	95.9	96.2	95.8	96.1	96.2	96.4	94.6	92.5

Table F3: Percentage fragment contents (SEC-HPLC) of Formulations 1-12 at different temperatures and times

	T=0	2-8 °C		-20 °C		25 °C			40 °C		
		2W	4W	2W	4W	1W	2W	4W	1W	2W	4W
Formulation 1	0.2	0.2	0.0	0.2	0.0	0.2	0.3	0.1	0.3	0.4	0.5
Formulation 2	0.2	0.1	0.0	0.0	0.0	0.2	0.2	0.1	0.2	0.2	0.4
Formulation 3	0.2	0.2	0.0	0.0	0.0	0.2	0.2	0.1	0.2	0.3	0.4
Formulation 4	0.1	0.2	0.0	0.0	0.0	0.2	0.2	0.2	0.2	0.3	0.7
Formulation 5	0.2	0.1	0.0	0.2	0.0	0.2	0.2	0.2	0.2	0.4	0.5
Formulation 6	0.1	0.0	0.0	0.2	0.0	0.2	0.2	0.2	0.2	0.3	0.5
Formulation 7	0.2	0.2	0.0	0.2	0.0	0.2	0.2	0.1	0.3	0.4	0.5
Formulation 8	0.2	0.2	0.0	0.2	0.0	0.2	0.2	0.1	0.2	0.3	0.4
Formulation 9	0.2	0.2	0.0	0.2	0.0	0.2	0.0	0.1	0.3	0.3	0.4
Formulation 10	0.2	0.2	0.0	0.2	0.0	0.2	0.2	0.1	0.2	0.2	0.4
Formulation 11	0.2	0.2	0.0	0.0	0.0	0.2	0.2	0.1	0.2	0.2	0.4
Formulation 12	0.2	0.2	0.0	0.2	0.0	0.2	0.3	0.1	0.3	0.4	0.5

[00162] According to the CEX-HPLC results in Table G1, while the proportion of acidic components in Formulations 1-12 increased after being stored at high temperature (40 °C) for 4 weeks, the proportion of acidic components in Formulations 7-9 increased to a lesser degree compared to T=0.

[00163] According to the CEX-HPLC results in Table G2, while the proportion of main peaks in Formulations 1-12 decreased after being stored at high temperature (40 °C) for 4 weeks, the proportion of main peaks in Formulations 9 and 12 decreased to a lesser extent compared to T=0.

[00164] According to the CEX-HPLC results in Table G3, while the proportion of alkaline components in Formulations 1-12 increased after being stored at high temperature (40 °C) for 4 weeks, the proportion of alkaline components in Formulations 9 and 12 increased to a lesser extent compared to T=0.

[00165] These results show that there is a significant correlation between the ADC charge components and pH of the formulation. When pH is below 6.0, the acidic and alkaline components increase rapidly, while when pH is above 6.0, the acidic and alkaline components increase relatively slowly. Under the conditions of 2-8 °C and -20 °C, there was no significant change in the acidic component, alkaline component, and main peak amounts.

Table G1: Content of acidic peaks (CEX-HPLC) of Formulations 1-12 at different temperatures and times

	T=0	2-8 °C		-20 °C		25 °C			40 °C		
		2W	4W	2W	4W	1W	2W	4W	1W	2W	4W
Formulation 1	33.9	34.3	33.5	34.3	33.7	33.8	34.6	36.1	34.9	37.1	39.3
Formulation 2	34.7	35.1	34.7	35.5	34.6	34.6	35.6	36.6	36.2	38.1	41.0
Formulation 3	35.5	34.8	35.4	36.2	35.2	35.7	36.6	37.1	36.7	38.8	42.0
Formulation 4	34.0	34.1	34.5	34.1	34.7	33.8	35.1	35.8	36.7	40.5	45.2
Formulation 5	34.0	35.3	35.6	35.5	36.1	34.9	36.8	37.0	37.6	40.8	44.0
Formulation 6	37.0	39.2	38.0	39.5	38.6	38.2	40.1	38.7	39.9	42.3	43.5
Formulation 7	36.1	36.4	35.7	36.7	36.1	36.0	36.4	35.8	36.5	38.3	40.1
Formulation 8	39.5	39.7	38.2	39.9	39.9	39.4	39.6	39.2	39.6	40.7	42.5
Formulation 9	45.3	45.7	44.3	45.3	45.2	45.4	44.5	45.1	45.1	45.4	46.8
Formulation 10	35.8	36.2	36.3	36.4	36.7	36.7	37.7	37.3	38.1	40.6	42.6
Formulation 11	38.9	39.7	38.9	40.0	39.3	39.1	40.7	39.4	40.4	42.4	43.0
Formulation 12	43.0	44.1	42.9	44.4	43.2	43.6	45.1	43.4	44.8	46.4	46.3

Table G2: Content of main peaks (CEX-HPLC) of Formulations 1-12 at different temperatures and times

	T=0	2-8 °C		-20 °C		25 °C			40 °C		
		2W	4W	2W	4W	1W	2W	4W	1W	2W	4W
Formulation 1	36.5	36.2	37.1	36.3	37.1	35.8	34.8	33.1	32.8	28.7	22.6
Formulation 2	36.1	35.5	36.4	35.6	36.7	35.5	34.7	33.4	33.2	29.3	23.9
Formulation 3	35.6	36.1	36.0	35.1	36.3	29.4	29.3	33.3	33.3	29.3	24.3
Formulation 4	35.8	36.3	36.5	36.1	36.4	35.9	35.0	34.3	32.4	27.8	21.3
Formulation 5	35.5	35.0	35.7	35.2	35.2	35.2	34.0	33.8	32.5	28.5	23.2
Formulation 6	33.8	32.7	34.1	32.3	33.6	33.0	32.0	33.0	31.4	28.2	23.9
Formulation 7	36.2	36.0	36.8	35.9	36.3	35.6	35.0	34.7	33.8	29.6	24.3
Formulation 8	33.8	33.6	35.1	33.6	33.6	33.6	32.9	33.1	32.3	29.3	24.8
Formulation 9	29.6	29.4	30.6	29.6	29.9	29.4	29.9	29.0	29.1	27.5	23.5
Formulation 10	35.8	34.8	35.4	34.8	35	34.1	33.5	33.9	32.5	29.2	24.3
Formulation 11	32.6	31.8	33.4	32.0	33.1	32.6	31.7	32.6	31.1	28.5	24.8
Formulation 12	29.0	28.3	30.2	28.6	29.9	29.1	28.3	29.6	27.8	27.6	23.6

Table G3: Content of alkaline peaks (CEX-HPLC) of Formulations 1-12 at different temperatures and times

	T=0	2-8 °C		-20 °C		25 °C			40 °C		
		2W	4W	2W	4W	1W	2W	4W	1W	2W	4W
Formulation 1	29.6	29.7	29.5	29.4	29.2	30.4	30.6	30.8	32.3	34.2	38.1
Formulation 2	29.1	29.4	28.9	28.9	28.8	29.9	29.8	30.1	30.7	32.5	35.1
Formulation 3	28.9	29.1	28.6	28.8	28.6	34.9	34.1	29.6	30.0	31.9	33.7
Formulation 4	30.2	29.6	29.0	29.8	28.9	30.3	29.9	29.9	30.9	31.7	33.5
Formulation 5	30.4	29.7	28.7	29.3	28.7	29.9	29.1	29.2	29.9	30.7	32.8
Formulation 6	29.3	28.2	27.8	28.2	27.8	28.8	27.9	28.3	28.7	29.5	32.7
Formulation 7	27.7	27.7	27.6	27.4	27.6	28.4	28.7	29.4	29.8	32.2	35.7
Formulation 8	26.7	26.7	26.7	26.5	26.5	27.0	27.4	27.8	28.0	30.0	32.7
Formulation 9	25.0	24.9	25.1	25.1	25.0	25.1	25.6	25.8	25.8	27.1	29.7
Formulation 10	28.4	28.9	28.4	28.9	28.4	29.2	28.8	28.8	29.5	30.3	33.1
Formulation 11	28.4	28.6	27.7	28.0	27.5	28.3	27.6	28.1	28.5	29.0	32.1
Formulation 12	28.0	27.6	26.9	27.0	26.9	27.3	26.6	27.0	27.5	29.6	30.1

[00166] According to the R-CE-SDS purity results in Table H1, the purity of each formulation did not significantly decrease under accelerated and high-temperature conditions. After being placed under long-term conditions of 2-8 °C and -20 °C for 2 weeks, the purity of the samples did not change significantly, showing good stability.

Table H1: Purity (R-CE-SDS) of Formulations 1-12 at different temperatures and times

	T=0	2-8 °C		-20 °C		25 °C		40 °C	
		2W	4W	2W	4W	1W	2W	1W	2W
Formulation 1	96.2	96.7		96.8		96.5	95.9	96.5	95.3
Formulation 2	96.2	95.7		96.1		96.3	94.9	96.1	96.4
Formulation 3	95.8	95.8		96.4		96.2	96.4	95.8	96.6
Formulation 4	96.1	97.0		96.6		95.7	95.3	97.0	95.6
Formulation 5	96.0	96.3		96.8		96.6	95.1	96.9	95.6
Formulation 6	95.8	96.1		95.5		95.6	95.6	96.9	94.9
Formulation 7	96.4	96.1		95.7		96.3	96.2	96.9	94.4
Formulation 8	96.7	96.4		96.4		96.8	97.2	96.6	95.1
Formulation 9	96.3	96.2		96.0		96.2	96.1	96.6	96.1
Formulation 10	96.5	96.9		97.5		97.0	95.1	97.0	95.0
Formulation 11	96.2	96.5		97.0		95.9	95.2	96.9	95.5
Formulation 12	96.5	96.0		96.9		96.1	94.4	96.9	96.2

[00167] According to the NR-CE-SDS purity results in Table H2, after being placed at high temperature for 4 weeks, the purity of all samples decreased to a certain extent, but there was no significant difference in the decreasing trend of the purity of Formulations 1-12.

Table H2: Purity (NR-CE-SDS) of Formulations 1-12 at different temperatures and times

	T=0	2-8°C		20°C		25°C			40°C		
		2W	4W	2W	4W	1W	2W	4W	1W	2W	4W
Formulation 1	96.4	96.3	96.1	96.5	94.6	96.1	96.9	96.3	95.9	95.8	93.9
Formulation 2	96.3	97.2	96.6	96.6	97.0	96.3	96.7	96.4	96.8	96.3	95.5
Formulation 3	96.3	96.5	96.1	96.6	97.2	96.1	94.8	96.5	96.3	96.0	95.2
Formulation 4	96.3	96.8	96.3	96.7	97.4	96.5	96.9	95.8	96.4	96.1	94.3
Formulation 5	96.2	96.8	96.7	97.1	97.8	96.7	95.5	96.0	96.6	96.2	94.1
Formulation 6	96.4	96.7	96.5	97.1	97.4	96.3	96.5	96.2	96.8	95.6	94.5
Formulation 7	96.0	96.8	96.5	96.8	97.3	95.8	96.9	96.2	96.2	95.2	94.5
Formulation 8	96.4	96.8	96.5	96.5	96.9	96.3	96.4	96.2	96.2	95.4	94.8
Formulation 9	96.4	96.9	96.5	96.6	97.0	96.2	96.5	96.1	96.3	95.8	93.6
Formulation 10	96.2	96.9	96.5	96.2	97.8	95.9	96.7	96.8	96.2	96.0	94.0
Formulation 11	96.2	97.2	96.5	96.7	97.3	95.8	96.7	95.7	96.5	95.7	94.6
Formulation 12	96.1	97.2	96.7	96.7	97.5	96.2	96.6	96.8	97.1	95.2	94.1

[00168] Analysis of the DAR values of Formulations 1-12, indicated that there were no significant changes from the initial value of 3.5 under any of the different storage conditions, showing good stability.

[00169] Table I1 shows that at 2-8°C and -20°C the Linker-Payload falls off relatively slowly. Under the conditions of 20°C and 40°C, the Linker-Payload fell off relatively quickly, but there was no significant difference in the shedding rate of the Linker-Payload among Formulations 1-12. However, the shedding rate of Linker-Payload is clearly related to the pH value of the buffer. A low pH value will accelerate the shedding of Linker-Payload, while a higher pH value can slow down the shedding of Linker-Payload.

Table I1: Content of Linker-Payload (mg/ml) of Formulations 1-12 at different temperatures and times

	T=0	2-8°C		20°C		25°C			40°C		
		2W	4W	2W	4W	1W	2W	4W	1W	2W	4W
Formulation 1	<1.0	<1.0	1.5	<1.0	<1.0	2.2	2.1	2.8	2.4	5.5	7.9
Formulation 2	<1.0	1.0	2.2	<1.0	<1.0	1.3	1.8	3.2	1.9	3.3	8.8
Formulation 3	<1.0	1.6	1.8	<1.0	1.0	<1.0	2.3	3.6	2.3	5.2	9.1
Formulation 4	<1.0	<1.0	2.6	<1.0	1.1	1.8	3.9	4.2	2.8	9.0	9.0
Formulation 5	<1.0	<1.0	1.7	<1.0	1.1	1.6	2.0	3.7	2.3	5.8	8.7
Formulation 6	<1.0	1.3	2.0	<1.0	1.4	1.4	2.3	3.5	2.1	4.9	8.3
Formulation 7	<1.0	1.1	2.0	<1.0	1.0	<1.0	2.5	3.4	3.6	4.7	9.8
Formulation 8	<1.0	1.4	2.1	<1.0	<1.0	1.5	2.8	3.1	2.0	4.8	9.1
Formulation 9	<1.0	1.6	2.4	<1.0	1.2	1.9	2.0	3.6	2.8	4.7	8.1
Formulation 10	<1.0	1.3	1.8	<1.0	1.0	1.3	2.9	4.6	2.1	3.7	8.8
Formulation 11	1.4	1.1	2.3	<1.0	<1.0	1.6	4.0	5.0	1.8	5.2	7.6
Formulation 12	<1.0	1.4	2.8	<1.0	1.1	1.3	3.4	5.6	1.9	4.1	6.7

[00170] The data in Table I2 show a similar trend; the free drug content of the formulations increases at higher temperatures and lower pH values.

Table I2: Content of Free Drug (mg/ml) of Formulations 1-12 at different temperatures and times

	T=0	2-8°C		20°C		25°C			40°C		
		2W	4W	2W	4W	1W	2W	4W	1W	2W	4W
Formulation 1	0.4	0.4	0.3	0.4	0.3	1.0	1.8	2.4	2.7	8.6	14.3
Formulation 2	0.2	0.5	0.3	0.4	0.2	0.6	0.9	1.6	1.6	4.3	8.5
Formulation 3	0.2	0.6	0.2	0.2	0.2	0.5	0.9	1.1	1.1	3.7	6.1
Formulation 4	0.4	0.7	0.3	0.4	0.4	0.6	1.4	1.8	1.9	8.1	11.6
Formulation 5	0.2	0.7	0.2	0.3	0.4	0.4	0.5	1.0	1.0	3.8	5.5
Formulation 6	0.2	0.7	0.3	0.3	0.2	0.3	0.7	0.6	0.8	2.4	3.5
Formulation 7	0.3	0.5	0.2	0.3	0.2	0.4	0.9	1.3	1.7	4.5	9.1
Formulation 8	0.2	0.3	0.3	0.3	0.3	0.4	0.5	0.9	1.1	3.2	5.7
Formulation 9	0.2	0.4	0.2	0.3	0.2	0.4	0.4	0.6	0.8	2.0	4.7
Formulation 10	0.3	0.3	0.3	0.3	0.2	0.5	0.7	1.2	1.0	2.7	5.2
Formulation 11	0.2	0.3	0.3	0.3	<0.2	0.4	0.6	0.8	0.7	2.0	3.5
Formulation 12	0.3	0.4	0.7	0.2	0.3	0.4	0.7	1.0	0.6	1.8	3.2

[00171] Table J shows the binding activity test results as determined by ELISA of Formulations 7 to 9 after storage under different conditions. This data shows that the binding activity of the ADC in the histidine buffer system does not significantly change after being placed for 4 weeks in the pH range of 5.4 to 6.2 at all temperatures investigated.

Table J: Percentage binding activity (ELISA) test results of Formulations 7-9 at different temperatures and times

	T=0	2-8 °C		20 °C		25 °C		40 °C	
		1W	4W	1W	4W	1W	4W	1W	4W
Formulation 7	104	113	114	98	107	106	108	97	102
Formulation 8	101	88	107	83	108	84	98	91	110
Formulation 9	93	90	81	104	96	88	86	104	94

3.4 Conclusions of Initial Formulation Screening

[00172] On the basis of visible foreign particles, insoluble particles and SEC analysis results, ADC is more likely to form aggregates and particles in citrate buffer and succinate buffers. In addition, the SEC results show that under the same buffer system, the higher the pH value, the faster the rate of aggregate formation.

[00173] The CEX test results show that when the pH is lower than 6.0, the acidic components and basic components increase rapidly. When the pH is higher than 6.0, the acidic components and basic components increase more slowly.

[00174] Free Drug analysis shows that the shedding rate of payload is obviously related to the pH value of the buffer. A low pH value will accelerate the shedding of payload, and a higher pH value can slow down the shedding of payload. The pH range of acetate buffer (pH 4.8 to 5.4) is relatively low.

[00175] In summary, based on the results of the first round of formulation screening, 20 mM histidine buffer with a pH range of 5.8 ± 0.3 (pH 5.5 to 6.1) was selected as the buffer system for the next round of screening experiments.

Example 4: Further Formulation Screening

4.1 Formation of ADC buffer formulations 13-24

[00176] 12 further buffer formulations according to Table K were prepared. A dialysis bag was soaked in the respective buffer solution, which also contained a stabilizer as indicated in Table K, one end was sealed with a dialysis clip, ADC sample (37.4 mg/ml) was transferred carefully to the dialysis bag, and the bag mouth was clamped. The bag was placed in a beaker filled with the buffer solution according to Table K, and dialysis was performed at room temperature with magnetic stirring and two changes of the fluid. After room temperature dialysis was completed, the dialysis bag was transferred to a fresh dialysis solution and overnight dialysis at 2-8 °C was carried out. During each dialysis fluid replacement process, the volume of the replacement fluid did not exceed 1:100. After dialysis, the protein concentration, and pH value were measured and the samples were diluted with the corresponding buffer to a target concentration of 20.0 mg/ml. Finally, freshly prepared surfactant (10% PS 20 or PS 80) was added to each formulation to the level specified in Table K.

[00177] The formulations 13-24 were aseptically filled into 2R vials (1.0 ml/bottle) on a clean bench and plugged and capped. Formulations 13-24 were then stored under various conditions (-20 °C, 2-8 °C, 25 °C, and 40 °C) for up to 3 months and analysed periodically as described in section 4.2.

[00178] Formulations 13-24 were also subjected to freeze-thaw cycling (3 cycles or 5 cycles), shaking for up to 5 days, and illumination for up to 10 days. The freeze-thaw cycling was performed using a Refrigerator at -20°C, (Haier, DW-25L262). The shaking was performed at 25 °C, 300rpm using a constant temperature shaker (Hrystal, IS-RDS3). The illumination testing was performed at 4500±500 lux in a Light test chamber (Binder, KBF P 240(E6)).

Table K: Summary of further formulations

Formulation	Buffer	Concentration of protein in ADC	Stabilizer	Surfactant
13	20 mM Histidine buffer, pH 5.8	20 mg/ml	6% (w:v) Sucrose	0.03% (w:v) PS 20
14	20 mM Histidine buffer, pH 5.8		6% (w:v) Sucrose	0.03% (w:v) PS 80
15	20 mM Histidine buffer, pH 5.8		6% (w:v) Trehalose	0.03% (w:v) PS 20
16	20 mM Histidine buffer, pH 5.8		6% (w:v) Trehalose	0.03% (w:v) PS 80
17	20 mM Histidine buffer, pH 5.5		6% (w:v) Sucrose	0.03% (w:v) PS 20
18	20 mM Histidine buffer, pH 5.5		6% (w:v) Trehalose	0.03% (w:v) PS 20
19	20 mM Histidine buffer, pH 5.5		6% (w:v) Sucrose	0.03% (w:v) PS 80
20	20 mM Histidine buffer, pH 5.5		6% (w:v) Trehalose	0.03% (w:v) PS 80
21	20 mM Histidine buffer, pH 6.1		6% (w:v) Sucrose	0.03% (w:v) PS 20
22	20 mM Histidine buffer, pH 6.1		6% (w:v) Trehalose	0.03% (w:v) PS 20
23	20 mM Histidine buffer, pH 6.1		6% (w:v) Sucrose	0.03% (w:v) PS 80
24	20 mM Histidine buffer, pH 6.1		6% (w:v) Trehalose	0.03% (w:v) PS 80

4.2 Analysis of ADC buffer formulations 1-12

[00179] Protein concentration, insoluble particle levels, % aggregates/monomers/fragments by SEC-HPLC, charge heterogeneity by CEX-HPLC, purity by R-CE-SDS/NR-CE-SDS, DAR, free drug levels, & binding activity by ELISA were determined for Formulations 13-24 according to the analysis methods described in the General Methods section above.

[00180] Osmotic pressure of the samples was determined using an Osmomat 3000 osmotic pressure detector. Before sample detection, a calibration solution (300 mOsm/L, 500 mOsm/L) was used.

4.3 Results of Initial Formulation Screening

[00181] The protein concentration, pH, and osmotic pressure of the buffer formulations after the various treatments (where F/T = freeze-thaw; S = shaking; I = illumination) are shown in Tables L, M, and N respectively. Except for the samples subjected to 10 days illumination, which showed increased protein concentrations and lowered pH values, there were no significant variations in these parameters after the different treatments.

Table L: Protein concentration (mg/ml) of Formulations 13-24 (ND = not determined)

Table E: Protein Concentration (mg/ml) of Formulations 13-24 (ND = not determined)															
Formulation	T=0	40°C			25°C			-20°C			-2-8°C		F/T	S	I
		2W	4W		2W	4W	3M	4W	3M		4W	3M			
13	20.2	20.2	20.2	20.3	20.3	21.6	20.2	21.0	20.1	20.9	20.3	20.3	22.0		
14	20.0	20.0	19.8	20.0	19.9	20.8	19.9	20.7	19.9	20.7	19.9	20.0	21.7		
15	20.0	19.9	19.9	20.0	19.9	ND	19.9	ND	19.9	ND	19.8	19.9	21.4		
16	20.7	20.5	20.6	20.6	20.6	ND	20.6	ND	20.6	ND	20.6	20.7	22.4		
17	20.3	20.5	20.6	20.4	20.3	ND	ND	ND	ND	ND	ND	ND	ND		
18	20.0	20.1	20.1	20.2	20.2	ND	ND	ND	ND	ND	ND	ND	ND		
19	20.9	20.8	20.7	20.8	20.7	ND	ND	ND	ND	ND	ND	ND	ND		
20	20.3	20.0	20.1	20.0	20.0	ND	ND	ND	ND	ND	ND	ND	ND		
21	20.2	20.1	20.3	20.0	20.1	20.9	ND	ND	ND	ND	ND	ND	ND		
22	20.2	20.1	20.1	20.3	20.2	20.6	ND	ND	ND	ND	ND	ND	ND		
23	20.4	20.5	20.5	20.5	20.5	21.3	ND	ND	ND	ND	ND	ND	ND		
24	20.0	19.7	19.6	19.6	19.8	20.3	ND	ND	ND	ND	ND	ND	ND		

Table M: pH of Formulations 13-24 (ND = not determined)

Table IV: PI of Formulations 13-24 (ND = not determined)													
Formulation	T=0	40°C		25°C			-20°C		-2-8°C		F/T	S	I
		2W	4W	2W	4W	3M	4W	3M	4W	3M	5C	5D	10D
13	5.80	5.79	5.79	5.79	5.80	ND	5.78	ND	5.79	ND	5.78	5.76	4.59
14	5.80	5.78	5.79	5.79	5.79	ND	5.79	ND	5.80	ND	5.79	5.78	4.56
15	5.80	5.80	5.78	5.80	5.78	ND	5.79	ND	5.79	ND	5.79	5.77	4.57
16	5.80	5.79	5.80	5.78	5.79	ND	5.78	ND	5.78	ND	5.79	5.78	4.59
17	5.50	5.51	5.51	5.50	5.51	ND	ND	ND	ND	ND	ND	ND	ND
18	5.50	5.50	5.50	5.49	5.50	ND	ND	ND	ND	ND	ND	ND	ND
19	5.50	5.49	5.49	5.50	5.50	ND	ND	ND	ND	ND	ND	ND	ND
20	5.50	5.50	5.50	5.51	5.52	ND	ND	ND	ND	ND	ND	ND	ND
21	6.10	6.09	6.09	6.10	6.09	ND	ND	ND	ND	ND	ND	ND	ND
22	6.10	6.10	6.10	6.09	6.10	ND	ND	ND	ND	ND	ND	ND	ND
23	6.10	6.09	6.10	6.10	6.09	ND	ND	ND	ND	ND	ND	ND	ND
24	6.10	6.10	6.08	6.09	6.10	ND	ND	ND	ND	ND	ND	ND	ND

Table N: Osmotic pressure (mOsm/L) of Formulations 13-24 (ND = not determined)

TABLE 1. Smoke Measurements (700-500/27 of 40 minutes) at 10, 25, and 200 °C (ND = not determined)														
Formulation	T=0	40°C		25°C			-20°C			-2-8°C		F/T	S	I
		2W	4W	2W	4W	3M	4W	3M	4W	3M	5C	5D	10D	
13	222	222	221	221	222	ND	222	ND	221	ND	221	221	222	
14	219	220	220	220	221	ND	219	ND	220	ND	218	221	223	
15	203	204	205	203	203	ND	204	ND	203	ND	203	203	204	
16	203	203	204	203	204	ND	203	ND	204	ND	202	203	203	
17	218	219	220	219	220	ND	ND	ND	ND	ND	ND	ND	ND	
18	206	206	205	206	206	ND	ND	ND	ND	ND	ND	ND	ND	
19	221	220	220	221	221	ND	ND	ND	ND	ND	ND	ND	ND	
20	206	207	208	206	205	ND	ND	ND	ND	ND	ND	ND	ND	
21	216	216	216	216	217	ND	ND	ND	ND	ND	ND	ND	ND	
22	206	206	206	206	205	ND	ND	ND	ND	ND	ND	ND	ND	
23	216	216	218	216	215	ND	ND	ND	ND	ND	ND	ND	ND	
24	206	207	208	206	207	ND	ND	ND	ND	ND	ND	ND	ND	

[00182] In terms of appearance of the formulations, no obvious visible foreign matter was observed in any of the samples at T=0 or after the above-described treatments. All samples were colourless clear, or slightly opalescent, liquids, apart from the sample subjected to 10 days of illumination which turned yellowish brown.

[00183] As shown in Tables O1-O3, the insoluble particle levels in the samples at different time points under different inspection conditions for Formulations 13-24 are relatively low. As seen in Table O1, comparing the particles levels at T=0 and after repeated freeze-thaw cycling and illumination,

formulation 14 performed slightly better than formulation 13. In terms of freeze-thaw cycling and shaking, formulation 14 performed slightly better than formulation 16. The only difference between formulation 14 and formulation 13 is the surfactant component (surfactant in formulation 14 is 0.03% polysorbate 80; surfactant in formulation 13 is 0.03% polysorbate 20) and the only difference between formulation 14 and formulation 16 is the stabilizer component (stabilizer in formulation 14 is 6% sucrose; stabilizer in formulation 16 is 6% trehalose). It appears that polysorbate 80 and sucrose can inhibit the generation of particulate particles in the ADC formulation.

Table O1: Insoluble particle levels following different treatments ($\geq 2\mu\text{m}$; $\geq 5\mu\text{m}$; $\geq 10\mu\text{m}$; $\geq 25\mu\text{m}$; unit = number/ml)

Formulation	T=0				Freeze-Thaw				Shake				Illumination			
					5 cycles				5 days				10 days			
	$\geq 2\mu\text{m}$	$\geq 5\mu\text{m}$	$\geq 10\mu\text{m}$	$\geq 25\mu\text{m}$	$\geq 2\mu\text{m}$	$\geq 5\mu\text{m}$	$\geq 10\mu\text{m}$	$\geq 25\mu\text{m}$	$\geq 2\mu\text{m}$	$\geq 5\mu\text{m}$	$\geq 10\mu\text{m}$	$\geq 25\mu\text{m}$	$\geq 2\mu\text{m}$	$\geq 5\mu\text{m}$	$\geq 10\mu\text{m}$	$\geq 25\mu\text{m}$
13	642	262	72	0	639	239	54	4	389	107	17	2	562	194	54	4
14	370	115	29	4	585	137	27	5	479	165	64	2	229	95	44	5
15	884	345	70	4	1260	409	72	4	2409	950	450	45	197	87	39	7
16	582	230	54	2	604	235	67	4	527	212	52	0	149	57	25	2

Table O2: Insoluble particle levels at different temperatures and times (A: $\geq 2\mu\text{m}$; B: $\geq 5\mu\text{m}$; unit: number/ml; * indicates no valid data obtained)

Form	T=0		2-8°C						20°C						25°C						40°C					
			2 W		4 W		3 M		2 W		4 W		3 M		2 W		4 W		3 M		2 W		4 W			
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
13	642	262	569	144	2745	812	*	*	549	119	760	299	253	60	570	245	1670	564	*	*	1167	487	582	239		
14	370	115	644	129	772	265	253	58	600	164	587	222	100	15	472	229	3260	437	948	218	709	345	1249	427		
15	884	345	2187	794	1387	462	ND	ND	1329	389	534	179	ND	ND	560	199	610	227	ND	ND	990	420	382	99		
16	582	230	1210	525	495	172	ND	ND	644	190	579	179	ND	ND	505	230	845	434	ND	ND	675	267	454	119		
17	419	160	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	594	249	764	360	ND	ND	737	279	3029	887		
18	504	170	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	952	355	827	469	ND	ND	622	240	535	174		
19	375	124	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	584	230	860	307	ND	ND	1005	484	1025	477		
20	440	139	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	677	280	759	374	ND	ND	1059	465	1167	502		
21	250	104	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	615	242	1090	500	679	159	629	254	974	397		
22	300	95	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	899	357	1062	375	*	*	604	247	625	232		
23	369	139	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	792	372	1047	444	*	*	879	374	570	284		
24	250	102	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	875	399	617	180	*	*	807	300	527	162		

Table O3: Insoluble particle levels at different temperatures and times (C: $\geq 10\mu\text{m}$; D: $\geq 25\mu\text{m}$; unit: number/ml; * indicates no valid data obtained)

Form	T=0		2-8°C						20°C						25°C						40°C							
			2 W		4 W		3 M		2 W		4 W		3 M		2 W		4 W		3 M		2 W		4 W					
	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D
13	72	0	29	2	164	7	*	*	30	0	72	4	13	0	65	2	147	10	*	*	94	2	75	4				
14	29	4	24	2	67	7	10	3	45	4	60	2	3	0	69	7	89	4	35	0	69	2	127	5				
15	70	4	214	9	125	17	ND	ND	77	5	42	4	ND	ND	74	7	45	5	ND	ND	90	2	30	0				
16	54	2	145	5	44	2	ND	ND	54	2	40	7	ND	ND	64	7	87	2	ND	ND	67	4	34	5				
17	59	5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	57	2	99	4	ND	ND	64	2	224	4				
18	42	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	80	2	142	4	ND	ND	44	2	54	0				
19	32	4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	72	2	70	2	ND	ND	107	4	114	2				
20	37	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	69	5	90	0	ND	ND	124	2	145	4				
21	44	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	74	4	100	4	30	3	82	4	117	17				
22	49	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	92	2	59	2	*	*	70	2	90	2				
23	54	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	84	2	112	4	*	*	115	5	70	2				
24	45	4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	107	4	64	4	*	*	75	0	62	2				

[00184] All formulations showed no significant differences in the proportion of polymers, monomers, and fragments under the different conditions. After being placed at 40 °C for 4 weeks, the proportion of polymers increased by 0.9%-1.2%, and the proportion of monomers decreased by 1.7%-2.0%. However, following light illumination, the proportion of aggregates significantly increased, while the proportion of monomers significantly decreased. The SEC-HPLC detection results are shown in Tables P1 to P3.

Table P1: Percentage aggregate contents (SEC-HPLC) of Formulations 13-24 under different conditions

Formulation	T=0	40°C			25°C				-20°C			2-8°C			F/T		S		I	
		1W	2W	4W	1W	2W	4W	3M	2W	4W	3M	2W	4W	3M	3C	5C	3D	5D	3D	10D
13	2.8	2.9	3.3	3.9	2.6	2.7	2.7	3.2	2.7	2.4	2.9	2.7	2.5	2.9	2.7	2.7	2.7	2.7	32.8	34.9
14	2.8	2.8	3.2	3.8	2.6	2.7	2.7	3.2	2.7	2.4	2.9	2.7	2.4	2.9	2.7	2.7	2.7	2.7	32.3	34.1
15	2.8	2.8	3.2	3.7	2.6	2.7	2.6	ND	2.6	2.4	ND	2.6	2.4	ND	2.7	2.7	2.6	2.6	33.5	35.2
16	2.8	2.8	3.2	3.8	2.6	2.7	2.7	ND	2.7	2.4	ND	2.7	2.5	ND	2.7	2.7	2.7	2.6	33.8	34.6
17	2.7	2.7	3.1	3.6	2.6	2.7	2.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
18	2.7	2.7	3.1	3.6	2.6	2.7	2.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
19	2.7	2.7	3.2	3.6	2.6	2.7	2.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
20	2.7	2.7	3.1	3.6	2.6	2.7	2.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
21	2.8	2.9	3.4	4.0	2.7	2.8	2.7	3.4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
22	2.8	2.9	3.4	4.0	2.7	2.8	2.7	3.4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
23	2.8	2.9	3.3	3.9	2.7	2.8	2.7	3.4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
24	2.8	2.9	3.3	3.9	2.7	2.6	2.7	3.4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table P2: Percentage monomer contents (SEC-HPLC) of Formulations 13-24 under different conditions

Formulation	T=0	40°C			25°C				-20°C			-24°C			F/T		S		I	
		1W	2W	4W	1W	2W	4W	3M	2W	4W	3M	2W	4W	3M	3C	5C	3D	5D	3D	10D
13	97.2	96.6	96.4	95.3	97.4	97.2	97.1	96.7	97.2	97.6	97.1	97.2	97.5	97.1	97.3	97.3	97.4	97.4	66.0	63.6
14	97.2	96.7	96.4	95.4	97.4	97.1	97.3	96.8	97.2	97.6	97.1	97.2	97.6	97.1	97.3	97.3	97.4	97.4	66.6	64.5
15	97.2	96.7	96.4	95.4	97.4	97.2	97.4	ND	97.3	97.6	ND	97.3	97.6	ND	97.3	97.3	97.4	97.4	65.4	63.4
16	97.2	96.6	96.4	95.4	97.4	97.1	97.3	ND	97.3	97.6	ND	97.2	97.6	ND	97.3	97.3	97.4	97.4	65.2	63.9
17	97.2	96.7	96.5	95.4	97.4	97.2	97.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
18	97.3	96.7	96.5	95.4	97.4	97.2	97.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
19	97.2	96.7	96.5	95.5	97.4	97.2	97.4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
20	97.2	96.7	96.5	95.4	97.4	97.2	97.4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
21	97.2	96.6	96.3	95.2	97.3	97.1	97.3	96.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
22	97.1	96.6	96.3	95.1	97.3	97.1	97.3	96.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
23	97.2	96.6	96.3	95.2	97.3	97.1	97.3	96.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
24	97.2	96.6	96.4	95.3	97.3	97.3	97.3	96.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table P3: Percentage fragment contents (SEC-HPLC) of Formulations 13-24 under different conditions

Formulation	T=0	40°C			25°C				-20°C			2-8°C			F/T		S		I	
		1W	2W	4W	1W	2W	4W	3M	2W	4W	3M	2W	4W	3M	3C	5C	3D	5D	3D	10D
13	0.1	0.5	0.4	0.8	0.0	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	1.2	1.4
14	0.1	0.5	0.4	0.8	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	1.1	1.4
15	0.0	0.5	0.4	0.8	0.0	0.1	0.0	ND	0.1	0.1	ND	0.0	0.0	ND	0.0	0.1	0.0	0.1	1.1	1.4
16	0.1	0.5	0.4	0.8	0.0	0.1	0.0	ND	0.0	0.0	ND	0.0	0.0	ND	0.0	0.0	0.0	0.0	1.1	1.5
17	0.1	0.6	0.4	1.0	0.0	0.1	0.3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
18	0.0	0.6	0.4	1.0	0.0	0.1	0.3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
19	0.0	0.6	0.4	1.0	0.0	0.1	0.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
20	0.1	0.6	0.4	1.0	0.0	0.1	0.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
21	0.1	0.5	0.4	0.9	0.0	0.1	0.0	0.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
22	0.1	0.5	0.4	0.9	0.0	0.1	0.0	0.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
23	0.1	0.5	0.4	0.9	0.0	0.1	0.0	0.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
24	0.1	0.5	0.4	0.9	0.0	0.1	0.0	0.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[00185] The charge heterogeneity (CEX-HPLC) results are shown in Tables Q1-Q3. After 4 weeks at 40°C (i) the proportion of the main peak of the formulations with pH 5.5 (Formulations 17-20) decreased by about 12% and the acidic and alkaline components increased by about 3.5% and 8.5% respectively; (ii) the proportion of the main peak of the formulations with pH 5.8 (Formulations 13-16) decreased by about 10%, the acidic and alkaline components increased by about 3% and 7% respectively; and (iii) the main peak proportion of the formulations with pH 6.1 (Formulations 21-24) decreased by about 8%, and the acidic and alkaline components increased by about 3% and 5% respectively. This shows that the ADC molecule is more stable in the pH 6.1 formulation. After 3 months of investigation at 25°C, the main peak also decreased to a certain extent, and the proportions of acidic components and alkaline components increased slightly. After 5 and 10 days of illumination, the CEX spectrum has changed

significantly (charge isomer analysis cannot be performed), indicating that the sample has been significantly damaged. Under other conditions, there was no significant change in the charge isomers of each system.

Table Q1: Content of acidic peaks (% by CEX-HPLC) of Formulations 13-24 under different conditions

Formulation	T=0	40°C			25°C				20°C			2-8°C			F/T		S		I	
		1W	2W	4W	1W	2W	4W	3M	2W	4W	3M	2W	4W	3M	3C	5C	3D	5D	5D	10D
13	38.7	40.5	41.8	41.3	38.4	40.0	39.1	39.7	40.2	38.6	39.1	40.6	38.7	39.1	38.1	39.0	39.6	38.8	N/A	N/A
14	38.6	40.4	40.0	41.4	38.3	40.1	37.9	39.5	40.8	38.7	39.2	40.6	39.0	39.2	37.9	39.0	39.4	39.1	N/A	N/A
15	37.8	41.9	40.0	41.4	38.2	40.3	38.2	ND	40.1	38.7	ND	40.7	38.9	ND	38.9	39.1	39.2	39.2	N/A	N/A
16	38.2	39.9	39.9	41.4	38.3	40.2	37.8	ND	39.9	38.4	ND	40.5	38.4	ND	37.8	39.0	38.9	38.9	N/A	N/A
17	35.8	39.8	40.3	39.5	35.6	38.0	35.7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
18	36.1	40.0	40.6	39.8	36.0	38.3	36.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
19	35.8	39.8	38.7	39.6	35.8	38.2	35.9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
20	35.9	39.9	38.7	39.6	35.9	38.3	36.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
21	41.6	43.3	43.3	44.4	42.2	42.4	41.6	43.9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
22	41.7	43.2	43.3	44.3	42.2	42.4	41.6	43.8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
23	41.9	43.7	43.7	45.1	42.6	42.6	42.1	44.8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
24	41.6	43.3	43.5	45.5	42.1	42.3	41.5	43.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table Q2: Content of main peaks (% by CEX-HPLC) of Formulations 13-24 under different conditions

Formulation	T=0	40°C			25°C				20°C			2-8°C			F/T		S		I	
		1W	2W	4W	1W	2W	4W	3M	2W	4W	3M	2W	4W	3M	3C	5C	3D	5D	5D	10D
13	34.1	30.6	31.6	24.3	33.5	36.4	33.1	29.1	33.6	33.6	32.8	33.6	34.0	32.9	34.4	33.3	33.4	33.4	N/A	N/A
14	34.0	31.0	29.4	24.3	33.4	36.3	33.5	28.9	33.2	33.4	32.8	33.5	33.5	32.5	34.4	33.2	33.3	33.0	N/A	N/A
15	35.0	33.3	29.5	24.3	33.5	36.2	33.0	ND	33.7	33.4	ND	33.4	33.4	ND	33.5	33.2	33.4	33.0	N/A	N/A
16	34.5	31.3	29.5	24.4	33.4	36.3	33.2	ND	33.9	33.5	ND	33.5	33.6	ND	34.5	33.3	33.5	33.2	N/A	N/A
17	36.2	33.7	31.5	23.9	35.2	37.5	34.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
18	36.1	33.7	31.6	24.0	34.9	37.3	33.8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
19	36.1	33.7	28.9	23.8	35.0	37.4	33.9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
20	36.0	33.7	29.4	23.8	34.9	37.3	33.7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
21	31.8	29.6	28.6	23.7	30.7	31.3	30.6	27.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
22	31.5	29.4	28.3	23.8	30.5	31.2	30.6	27.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
23	31.5	29.5	28.3	23.7	30.5	31.3	30.5	26.7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
24	31.6	29.8	28.6	24.1	30.7	31.5	30.8	27.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table Q3: Content of alkaline peaks (% by CEX-HPLC) of Formulations 13-24 under different conditions

Formulation	T=0	40°C			25°C				20°C			2-8°C			F/T		S		I	
		1W	2W	4W	1W	2W	4W	3M	2W	4W	3M	2W	4W	3M	3C	5C	3D	5D	5D	10D
13	27.2	29.0	26.6	34.4	28.1	23.6	27.8	31.2	26.1	27.3	28.1	25.8	27.9	28.0	27.5	27.7	27.0	27.8	N/A	N/A
14	27.4	28.6	30.6	34.3	28.2	23.6	28.6	31.6	26.1	27.6	28.0	26.0	27.9	28.3	27.7	27.8	27.3	27.8	N/A	N/A
15	27.2	24.9	30.5	34.3	28.3	23.5	28.8	ND	26.2	27.8	ND	25.9	27.9	ND	27.7	27.7	27.4	27.8	N/A	N/A
16	27.4	28.8	30.5	34.2	28.4	23.6	29.1	ND	26.2	28.0	ND	26.0	28.0	ND	27.8	27.8	27.6	27.9	N/A	N/A
17	28.0	26.5	28.2	36.6	29.2	24.5	30.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
18	27.9	26.3	27.9	36.3	29.1	24.4	30.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
19	28.1	26.5	32.4	36.6	29.2	24.5	30.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
20	28.1	26.5	31.9	36.5	29.2	24.5	30.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
21	26.7	27.2	28.2	31.9	27.1	26.3	27.8	29.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
22	26.8	27.4	28.4	31.9	27.3	26.4	27.9	29.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
23	26.6	26.9	28.0	31.3	27.0	26.1	27.4	28.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
24	26.8	27.0	27.9	30.4	27.1	26.3	27.7	28.9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[00186] The purity (NR CE-SDS) results are shown in Table R. After 5 days of illumination, the purity of the samples was significantly reduced. After 4 weeks at 40°C, the purity of the samples dropped slightly, but there was no significant difference in the trend for the 12 formulation samples. Under other inspection conditions, there was no significant change in sample purity.

Table R: Purity (NR-CE-SDS) of Formulations 13-24 under different conditions

Formulation	T=0	40 °C			25 °C			-20 °C			2-8 °C			F/T		S		I	
		1W	2W	4W	1W	2W	4W	3M	1W	2W	4W	3M	3C	5C	3D	5D	5D	10D	
13	96.9	96.9	95.6	97.3	96.9	96.2	95.3	97.0	97.2	95.6	96.6	97.4	96.4	96.9	97.0	96.5	69.9	68.7	
14	96.4	96.8	94.8	96.5	96.9	96.8	95.3	97.0	96.2	95.8	96.8	95.6	96.9	96.8	97.0	96.4	71.6	67.3	
15	96.7	96.6	95.5	96.0	96.9	ND	95.6	97.0	ND	96.8	96.9	ND	97.1	97.0	96.6	96.5	74.4	69.1	
16	96.6	96.5	94.7	96.9	97.2	ND	95.6	96.5	ND	96.6	96.8	ND	96.9	96.8	96.5	96.5	74.8	69.0	
17	96.5	96.7	95.3	96.7	96.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
18	96.5	96.4	95.5	96.6	97.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
19	96.7	96.5	95.4	96.8	96.8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
20	96.7	96.5	95.7	97.2	96.7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
21	96.6	96.3	95.4	96.8	97.0	96.4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
22	96.7	96.0	95.7	97.0	96.9	96.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
23	96.2	95.6	94.0	96.7	97.1	96.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
24	96.7	96.4	95.4	96.7	97.2	95.7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	

[00187] Analysis of the DAR values of Formulations 13-24, indicated that there were no significant changes from the initial value of 3.5 under any of the different storage conditions, apart from after 5-10 days of illumination, after which the HIC spectra were changed significantly so that DAR value analysis could not be performed, indicating that the sample had been significantly degraded.

[00188] Table S1 shows that at 2-8 °C and -20 °C, and after freeze-thaw cycling, the Linker-Payload falls off relatively slowly. Under the conditions of 25 °C and 40 °C, the Linker-Payload fell off relatively quickly, but there was no significant difference in the shedding rate of the Linker-Payload among Formulations 13-24, indicating that the different pH values and buffer systems investigated did not influence shedding.

Table S1: Content of Linker-Payload (mg/ml) of Formulations 13-24 under different conditions

Table S1. Content of Enkter Payload (mg/ml) of Formulations 13-24 under different conditions																					
Formulation	T=0	40°C			25°C				20°C				2-8°C			F/T		S		I	
		1W	2W	4W	1W	2W	4W	3M	3W	4W	3M	2W	4W	3M	3C	5C	3D	5D	5D	10D	
13	5.5	8.2	10.7	11.6	6.6	7.7	7.4	8.9	5.0	5.0	5.9	5.3	6.1	9.3	5.6	5.7	8.1	8.6	N/A	N/A	
14	4.9	9.3	9.6	9.9	6.3	7.0	6.9	10.4	5.0	5.4	6.5	4.9	5.9	5.5	5.4	5.8	6.3	7.3	N/A	N/A	
15	5.4	9.7	10.0	9.7	6.3	7.1	6.8	ND	4.6	4.7	ND	6.4	6.0	ND	5.5	5.4	6.6	7.9	N/A	N/A	
16	5.3	9.8	11.0	10.0	6.6	7.3	7.2	ND	5.0	5.1	ND	5.6	6.0	ND	5.9	5.8	7.4	7.3	N/A	N/A	
17	5.0	9.6	10.9	10.6	7.3	7.0	7.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
18	4.6	9.4	10.5	10.9	6.5	7.0	7.4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
19	5.0	9.1	10.5	10.2	6.4	7.4	7.9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
20	5.1	9.2	14.2	10.2	6.5	7.6	7.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
21	4.8	8.3	10.0	9.8	6.7	7.8	7.8	12.9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
22	4.9	8.4	9.4	9.3	7.3	7.8	7.9	14.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
23	5.5	8.6	10.7	9.8	7.0	7.7	7.8	13.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
24	5.4	7.6	9.3	9.2	6.3	7.0	7.6	11.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	

[00189] Table S2 shows the payload (free drug) falls off relatively slowly under conditions of 2-8 °C and -20 °C, freeze-thaw and shaking conditions, however, at 25 °C and 40 °C the Payload was shed more quickly. Under the 40 °C storage, the concentration of payload in the samples with pH 5.5 (formulations 17-20) and pH 5.8 (Formulations 13-16) increased significantly faster than the formulation with pH 6.1 (Formulations 21-24). This demonstrated that the payload in the ADC falls off more slowly under pH 6.1 conditions.

[00190] After 5 and 10 days of illumination, a large number of unknown impurity peaks appeared in the free drug detection spectra, indicating that the sample had been significantly degraded.

Table S2: Content of Free Drug (mg/ml) of Formulations 13-24 under different conditions

Table S2. Content of Flec Drug (mg/ml) of Formulations 13-24 under different conditions																				
Formulation	T=0	40°C			25°C				-20°C			2-8°C			F/T		S		I	
		1W	2W	4W	1W	2W	4W	3M	1W	2W	4W	3M	1W	2W	4W	3C	5C	3D	5D	5D
13	0.4	3.9	4.1	8.4	0.3	0.4	0.7	2.6	<0.2	0.2	0.2	<0.2	0.2	0.4	0.4	0.4	0.4	0.4	N/A	N/A
14	0.3	2.8	3.5	7.3	0.3	0.4	0.7	2.0	<0.2	0.2	<0.2	<0.2	0.2	<0.2	0.4	0.4	0.3	0.3	N/A	N/A
15	0.4	2.8	3.8	6.9	0.3	0.4	0.5	ND	<0.2	0.3	ND	0.2	0.3	ND	0.4	0.3	0.4	0.5	N/A	N/A
16	0.4	2.8	3.8	10.2	0.3	0.4	0.7	ND	0.3	0.3	ND	0.2	0.3	ND	0.4	0.4	0.4	0.4	N/A	N/A
17	0.3	3.9	5.4	7.6	0.6	0.8	1.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
18	0.3	3.5	5.0	10.9	0.4	0.7	1.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
19	0.3	3.6	5.2	9.9	0.4	0.8	1.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
20	0.4	3.5	7.0	10.2	0.4	0.9	1.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
21	0.3	1.9	2.9	5.4	0.4	0.5	0.4	3.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
22	0.3	1.9	2.6	5.0	0.4	0.4	0.5	3.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
23	0.4	1.9	3.2	5.2	0.4	<0.2	0.4	3.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
24	0.4	1.6	2.4	5.0	0.3	0.3	0.4	2.7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[00191] In terms of the binding activity measured by ELISA, after 5 and 10 days of illumination the relative binding activity of the samples tested dropped significantly (both below 60%), indicating that the sample has been significantly degraded. Under other inspection conditions, there was no significant change in the relative binding activity of the different formulation samples.

4.4 Conclusions of Further Formulation Screening

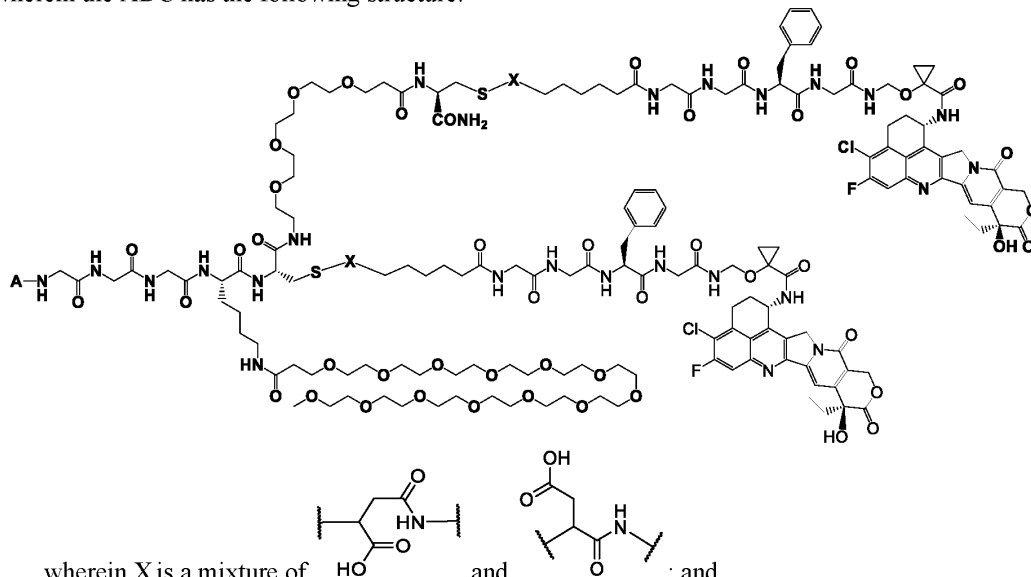
[00192] On the basis of visible foreign particles, SEC purity, non-reducing CE-SDS purity, DAR value, and relative binding activity, the results showed no significant differences between the 12 different Formulations. Insoluble particle analysis results showed that the surfactant polysorbate 80 is slightly better than polysorbate 20, and the stabilizer sucrose is slightly better than trehalose. Both CEX analysis results and free drug analysis results showed that the pH 6.1 buffer systems out-performed the pH 5.5 or pH 5.8 buffer systems.

[00193] Therefore, the further formulation screening determined that the preferred ADC formulation corresponded to Formulation 23 and comprised:

- i) 20mg/ml ADC (*active ingredient*);
- ii) 20mM histidine/histidine hydrochloride, pH 6.1 (*buffer*);
- iii) 6 % sucrose (W:V) (*stabilizer*); and
- iv) 0.03 % polysorbate 80 (W:V) (*surfactant*).

Claims

1. A pharmaceutical composition comprising an antibody drug conjugate (ADC) and a buffer, wherein the ADC has the following structure:



A is the anti-TROP2 antibody GQhRS7 or a fragment thereof;

wherein the linker and antibody or antibody fragment are connected by an amide bond to at least one light chain of the antibody or antibody fragment;

and wherein the pharmaceutical composition is a liquid and has a pH of 5.4 to 6.5.

2. The pharmaceutical composition of claim 1, wherein the linker and antibody or antibody fragment are connected by an amide bond at the C-terminus of at least one light chain of the antibody or antibody fragment.

3. The pharmaceutical composition of claim 1, wherein the linker and antibody or antibody fragment are connected by an amide bond at the C-terminus of each light chain of the antibody or antibody fragment.

4. The pharmaceutical composition according to any one of claims 1 to 3, wherein the ADC has an average drug to antibody ratio (DAR) in the range of 2 to 4, conveniently 3.5.

5. The pharmaceutical composition according to any one of claims 1 to 4, wherein the pharmaceutical composition is an aqueous liquid composition.

6. The pharmaceutical composition according to any one of claims 1 to 5, wherein the pharmaceutical composition is the form of an injectable aqueous solution.

7. The pharmaceutical composition according to claim 6, wherein the pharmaceutical composition is the form of an injectable aqueous solution for administration by intravenous infusion.

8. The pharmaceutical composition according to any one of claims 1 to 7, wherein the ADC is dissolved in the pharmaceutical composition at a concentration of 5 to 100 mg/mL, conveniently 5 to 50 mg/mL, more conveniently 15 to 25 mg/mL, yet more conveniently 20 mg/mL, wherein the concentration refers to the concentration of the protein of the ADC dissolved in the pharmaceutical composition.

9. The pharmaceutical composition according to any one of claims 1 to 8, wherein the pharmaceutical composition has a pH of 5.5 to 6.2, conveniently 5.8 to 6.2, more conveniently 6.0 to 6.2.

10. The pharmaceutical composition according to any one of claims 1 to 8, wherein the pharmaceutical composition has a pH of 6.1.

11. The pharmaceutical composition according to any one of claims 1 to 10, wherein the buffer is a histidine buffer.

12. The pharmaceutical composition according to claim 11, wherein the histidine buffer comprises a mixture of L-histidine and L-histidine monohydrochloride.

13. The pharmaceutical composition according to any one of claims 1 to 12, wherein the buffer components are present at a concentration of 5 to 50 mM, conveniently 10 to 30 mM, more conveniently 20 mM.

14. The pharmaceutical composition according to any one of claims 1 to 13, wherein the pharmaceutical composition further comprises a surfactant.

15. The pharmaceutical composition according to claim 14, wherein the surfactant is a polysorbate, conveniently polysorbate 80 or polysorbate 20, more conveniently polysorbate 80.

16. The pharmaceutical composition according to claim 14 or 15, wherein the pharmaceutical composition comprises 0.01 to 0.2 % w/v of the surfactant, conveniently 0.01 to 0.1 % w/v, more conveniently 0.02 to 0.05 % w/v, yet more conveniently 0.03 % w/v.

17. The pharmaceutical composition according to one of claims 1 to 16, wherein the pharmaceutical composition further comprises a stabiliser.

18. The pharmaceutical composition according to claim 17, wherein the stabiliser is a sugar stabiliser selected from the group including trehalose, mannitol, sucrose, maltose, lactose, fructose, xylitol, arabinol, erythritol, sylitol, sorbitol, raffinose, lactitol, maltitol, and inositol.

19. The pharmaceutical composition according to claim 17, wherein the stabiliser is a non-reducing sugar, conveniently selected from sucrose or trehalose, more conveniently sucrose.

20. The pharmaceutical composition according to any one of claims 17 to 19, wherein the pharmaceutical composition comprises 0.5 to 10 % w/v of the stabilizer, conveniently 3 to 8 % w/v, more conveniently 4 to 8 % w/v, yet more conveniently 6 % w/v.

21. The pharmaceutical composition according to any one of claims 1 to 20, wherein the pharmaceutical composition further comprises one or more one or more pharmaceutically acceptable excipients, such as a tonicity modifier, an antioxidant and/or a diluent.

22. The pharmaceutical composition according to any one of claims 1 to 21, wherein the osmolality of the pharmaceutical composition is between 150 and 450 mOSm/kg, conveniently between 200 and 400 mOSm/kg.

23. The pharmaceutical composition according to any one of claims 1 to 22, wherein the ADC is physically and/or chemically stable within the pharmaceutical composition for at least four weeks when stored at 2-8°C or -20°C.

24. The pharmaceutical composition according to any one of claims 1 to 23, wherein the ADC is stable within the pharmaceutical composition and has less than 5% aggregates as determined by SEC-HPLC when stored at 2-8°C or -20°C for at least four weeks.

25. The pharmaceutical composition according to any one of claims 1 to 24, wherein the pharmaceutical composition is the form of an injectable aqueous solution for administration by intravenous infusion and the solution is substantially or entirely free of particulate matter.

26. A process for manufacturing a pharmaceutical composition according to any one of claims 1 to 25, the process comprising the steps of mixing together the ADC and the buffer; and optionally any one or more additional components, optionally in any amount, concentration, or form; and optionally adjusting any one or more parameters, such as pH, in relation to the pharmaceutical composition.

27. A product obtainable by or obtained by the process of manufacturing a pharmaceutical composition as defined in claim 26.

28. A lyophilized formulation comprising the ADC and a buffer, wherein the formulation can be reconstituted to form the pharmaceutical composition according to any one of claims 1 to 25.

29. A method of preparing a lyophilized formulation, comprising lyophilizing the pharmaceutical composition according to any one of claims 1 to 25.

30. A container comprising a pharmaceutical composition according to any one of claims 1 to 25 or the lyophilized formulation according to claim 28, wherein the container can be a vial, single-use vial, light-protected vial, ampoule, syringe, pre-filled syringe, injection pen or intravenous infusion bag.

31. The pharmaceutical composition according to any one of claims 1 to 25 or the lyophilized formulation according to claim 28, for use as a medicament.

32. A method of treating cancer or an autoimmune disease in a subject in need thereof, the method comprises administering a therapeutically effective amount of the pharmaceutical composition according to any one of claims 1 to 25 or the lyophilized formulation according to claim 28.

33. A method of treating cancer in a subject in need thereof, the method comprises administering a therapeutically effective amount of the pharmaceutical composition according to any one of claims 1 to 25 or the lyophilized formulation according to claim 28.

34. The method according to claim 33, wherein the cancer is a TROP2-associated tumour.

35. The method according to claim 34, wherein the TROP2-associated tumour includes a tumour overexpressing TROP2 or a tumour with one or more TROP2 gene mutations.

36. The method according to any one of claims 30 to 35, wherein the cancer is selected from fibrosarcoma, myosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendothelial sarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon cancer, pancreatic cancer, breast cancer, thyroid cancer, endometrial cancer, melanoma, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous carcinoma, papillary carcinoma, papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, bronchial carcinoma, renal cell carcinoma, liver cancer, bile duct cancer, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung cancer, small cell lung cancer, bladder cancer, epithelial cancer, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma and retinoblastoma.

37. The method according to any one of claims 33 to 36 wherein the cancer is selected from breast cancer, gastric cancer, lung cancer, ovarian cancer, and urothelial cancer.

38. The method according to claim 32 or 37, wherein the subject is a mammal, preferably a human.

39. A kit of parts comprising a pharmaceutical composition according to any one of claims 1 to 25, or the lyophilized formulation according to claim 28, in a container and, optionally, a set of instructions with directions regarding the administration (e.g. by way of intravenous infusion) of the pharmaceutical composition or the lyophilized formulation.

40. A kit of parts comprising a lyophilized formulation according to claim 28 in a container, a diluent for reconstituting the lyophilized formulation and, optionally, a set of instructions with directions regarding the administration (e.g. by way of intravenous infusion) of the reconstituted formulation.