COMBINATION THERAPY WITH ANTIBODY-DRUG CONJUGATES

CONTINUITY

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 60/979,594, filed December 10, 2007; U.S. Provisional Patent Application No. 60/027,668, filed November 2, 2008; and U.S. Provisional Patent Application No. 61,040,641, filed March 28, 2008; the disclosures of which are incorporated by reference herein in their entirety.

FIELD

[0002] The present invention relates, *inter alia,* to methods for the treatment of Hodgkin lymphoma comprising administering both a chemotherapeutic regimen and an antibody-drug conjugate compound to a subject in need thereof.

BACKGROUND

[0003] Hodgkin lymphoma (HL) is a neoplasm of lymphoid tissue that is defined histopathologically by the presence of the malignant Hodgkin-Reed-Sternberg (HRS) cells. The characteristic surface antigen expressed on HRS cells is CD30. There are an estimated 8,000 new HL cases diagnosed annually in the United States and Canada. Advances in the use of combined chemotherapy and radiotherapy in HL over the past half-century have resulted in a durable remission rate of approximately 70%. However, these multi-agent regimens confer a significant morbidity on patients, including secondary malignancies, cardiac disease, and infertility. Furthermore, approximately 30% of patients presenting with HL will become refractory to initial therapy or will relapse. Salvage chemotherapy regimens and autologous stem cell transplant (ASCT) are secondary options for these patients, but both are associated with significant morbidity and limited long term disease control. Patients who relapse after ASCT or are ineligible for salvage therapy have a very poor prognosis. Currently, there is a lack of well-tolerated, efficacious treatment options for these patients.

[0004] Gemcitabine, alone or in combination with other chemotherapy, has been evaluated in the pre and post-ASCT setting. In the transplant naïve setting, relapsed or refractory HL patients treated with gemcitabine achieve response rates of 39% (Santoro et al., J Clin Oncol 2000 18(13):2615-9). In the relapsed/refractory setting where the majority of patients have received prior autologous or allogeneic transplant, gemcitabine response rates are diminished (22%) and hematologic toxicity of the regimen necessitates dose reduction to 1000 mg/m^2 (Venkatesh et al., Clin lymphoma 2004 5(2):110-5). A combination regimen utilizing gemcitabine, vinorelbine and pegylated liposomal doxorubicin (GVD) has demonstrated promising efficacy in relapsed/refractory HL. Overall response rates of 70% were observed in the combined analysis of pre and post-ASCT patients, however with dose limiting toxicities of mucositis in the pre-ASCT population and febrile neutropenia in the post-ASCT population (Bartlett al., CALGB 59804 Ann Oncoo, 2007 18(6): 1071-9). Only 32% and 26% of patients who were transplant naïve and post-ASCT, respectively, were able to receive all doses on schedule at full dose. For patients who do not respond to standard chemotherapy or who relapse, the only potentially curative therapy is high-dose chemotherapy in combination with stem cell transplantation. This treatment is also associated with significant morbidity and mortality, and a 5-year survival rate of less than 50%. Thus, there continues to be an unmet medical need for patients suffering from HL. The present invention addresses this and other needs.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] Figure 1A: Antitumor activity of cAC10-vcMMAE alone or in combination with ABVD on subcutaneous L540cy HL tumors in SCID mice. SCID mice were implanted with L540cy HL cells in the right flank. Groups of mice (9-10/group) were untreated or received cAC10-vcMMAE (1 mg/kg, q4dx3, ip) and/or ABVD: Adriamycin (1 mg/kg, q4dx3, i.v.), Bleomycin (7.5 u/kg, q4dx3, i.p.), Vinblastine (0.015 mg/kg, q4dx3, i.p.), and Dacarbazine (20 mg/kg, q3dx4, i.p.) when tumor size averaged approximately 100 mm^3.

[0006] Figure 1B: Antitumor activity of cAC10-vcMMAE alone or in combination with ABVD on subcutaneous L540cy HL tumors in SCID mice. SCID mice were implanted with L540cy HL cells in the right flank. Groups of mice (9-10/group) were untreated or received cAC10-vcMMAE (1 mg/kg, q4dx3, ip) and/or ABVD: Adriamycin (0.75 mg/kg, q4dx3, i.v.), Bleomycin (6 u/kg, q4dx3, i.p.), Vinblastine (0.01 mg/kg, q4dx3, i.p.), and Dacarbazine (15 mg/kg, q3dx4, i.p.) when tumor size averaged approximately 300 mm^3.

[0007] Figure 2A: Antitumor activity of cAC10-vcMMAE alone or in combination with Gemcitabine on subcutaneous L540cy HL tumors in SCID mice. SCID mice were implanted with L540cy HL cells in the right flank. Groups of mice (5-10/group) were untreated or received cAC10-vcMMAE (1 mg/kg, q4dx3, ip) alone, Gemcitabine (120 mg/kg, q4dx3, ip) alone, or combination treatment with cAC10-vcMMAE and Gemcitabine when tumor sizes averaged approximately 100 mm^3.

[0008] Figure 2B: Antitumor activity of cAC10-vcMMAE alone or in combination with Gemcitabine on subcutaneous L540cy HL tumors in SCID mice. SCID mice were implanted with L540cy HL cells in the right flank. Groups of mice (5-10/group) were untreated or received cAC10-vcMMAE (1 mg/kg, q4dx3, ip) alone, Gemcitabine (120 mg/kg, q4dx3, ip) alone, or combination treatment with cAC10-vcMMAE and Gemcitabine, when tumor sizes averaged approximately 300 mm^3.

[0009] Figure 3A: Antitumor activity of cAC10-vcMMAE alone or in combination with GVD on subcutaneous L540cy HL tumors in SCID mice. SCID mice were implanted with L540cy HL cells in the right flank. Groups of mice (8-10/group) were untreated or received cAC10-vcMMAE (1 mg/kg, q4dx3, ip) alone, GVD alone, or combination treatment with cAC10-vcMMAE and GVD when tumor sizes averaged approximately 100 mm^3. The treatment schedule of GVD was gemcitabine at 60 mg/kg q4dx3 ip, vinorelbine at 2 mg/kg q5dx3 ip, and doxorubicin at 1.5 mg/kg q4dx3 iv.

[0010] Figure 3B: Antitumor activity of cAC10-vcMMAE alone or in combination with GVD on subcutaneous L540cy HL tumors in SCID mice. SCID mice were implanted with L540cy HL cells in the right flank. Groups of mice (8-10/group) were untreated or received cAC10-vcMMAE (1 mg/kg, q4dx3, ip) alone, GVD alone, or combination treatment with cAC10-vcMMAE and GVD when tumor sizes averaged approximately 100 mm^3. The treatment schedule of GVD was gemcitabine at 60 mg/kg q4dx3 ip, vinorelbine at 2 mg/kg q5dx3 ip, and doxorubicin at 1.5 mg/kg q4dx3 iv.

[0011] Figure 4A: Antitumor activity of cAC10-vcMMAE alone or in combination with Vinorelbine on subcutaneous L540cy HL tumors in SCID mice. SCID mice were implanted with L540cy HL cells in the right flank. Groups of mice (5-10/group) were untreated or received cAC10-vcMMAE (1 mg/kg, q4dx3, ip) alone, Vinorelbine alone (4 mg/kg q5dx3), or combination treatment cAC10-vcMMAE and vinorelbine when tumor sizes averaged approximately 100 mm^3.

[0012] Figure 4B: Antitumor activity of cAC10-vcMMAE alone or in combination with Vinorelbine on subcutaneous L540cy HL tumors in SCID mice. SCID mice were implanted with L540cy HL cells in the right flank. Groups of mice (5-10/group) were untreated or received cAC10-vcMMAE (1 mg/kg, q4dx3, ip) alone, Vinorelbine alone (4 mg/kg q5dx3), or combination treatment cAC10-vcMMAE and vinorelbine when tumor sizes averaged approximately 100 mm^3.

[0013] Figure 5A: Antitumor activity of cAC10-vcMMAE alone or in combination with Doxorubicin on subcutaneous L540cy HL tumors in SCID mice. SCID mice were implanted with L540cy HL cells in the right flank. Groups of mice (5-10/group) were untreated or received cAC10-vcMMAE (1 mg/kg, q4dx3, ip) alone, Doxorubicin alone (3 mg/kg q4dx3), or combination treatment with cAC10-vcMMAE and doxorubicin when tumor sizes averaged approximately 100 mm^3.

[0014] Figure 5B: Antitumor activity of cAC10-vcMMAE alone or in combination with Doxorubicin on subcutaneous L540cy HL tumors in SCID mice. SCID mice were implanted with L540cy HL cells in the right flank. Groups of mice (5-10/group) were untreated or received cAC10-vcMMAE (1 mg/kg, q4dx3, ip)

alone, Doxorubicin alone (1.5 mg/kg q4dx3), or combination treatment with cAC10-vcMMAE and doxorubicin when tumor sizes averaged approximately 100 mm^3.

[0015] Figure 6: Antitumor activity of cAC10-vcMMAE alone or in combination with Vinblastine on subcutaneous L540cy HL tumors in SCID mice. SCID mice were implanted with L540cy HL cells in the right flank. Groups of mice (7-10/group) were untreated or received cAC10-vcMMAE (1 mg/kg, q4dx3, ip) alone, Vinblastine alone (0/1 mg/kg q4dx3), or combination treatment with cAC10-vcMMAE and vinblastine when tumor sizes averaged approximately 300 mm^3.

[0016] Figure 7: Antitumor activity of cAC10-vcMMAE alone or in combination with Gemcitabine on subcutaneous L540cy HL tumors in SCID mice. SCID mice were implanted with L540cy HL cells in the right flank. Groups of mice (6-8/group) were untreated or received cAC10-vcMMAE (1 mg/kg, q4dx3, ip) alone, Gemcitabine (120 mg/kg, q4dx3, ip) alone, or combination treatment with cAC10-vcMMAE and Gemcitabine when tumor sizes averaged approximately 100 mm^3.

[0017] Figure 8: Antitumor activity of cAC10-mcMMAF alone or in combination with Gemcitabine on subcutaneous L540cy HL tumors in SCID mice. SCID mice were implanted with L540cy HL cells in the right flank. Groups of mice (10/group) were untreated or received cAC10-mcMMAF (1 mg/kg, q4dx3, ip) alone, Gemcitabine (120 mg/kg, q4dx3, ip) alone, or combination treatment with cAC10-mcMMAF and Gemcitabine when tumor sizes averaged approximately 100 mm^3.

Figure 9: Altered Dosage Schedule for cAC10-vcMMAE. The total amount of cAC10-vcMMAE was kept constant at 3 mg/kg and the dose was split to various schedules.

DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS

A. General Introduction

[0018] The present invention provides, *inter alia,* methods for treating Hodgkin lymphoma. The present inventors have discovered that combination therapy with two different classes of anticancer compounds, antibody-drug conjugate compounds and chemotherapeutic agents, can improve a therapeutic benefit for subjects suffering from HL. In particular, the present inventors have found that combination therapy with either gemcitabine or an ABVD regimen and an anti-CD30 antibody conjugated to an auristatin compound provides synergistic therapeutic effects in the treatment of HL. Before the advent of the present invention, it could not have been expected that a chemotherapeutic agent and an anti-CD30 antibody conjugated to an auristatin compound would have a synergistic effect in the treatment of HL.

[0019] For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the subsections which follow.

B. Summary

[0020] The present invention is based, *inter alia,* on the discovery that combination therapy with either gemcitabine or an ABVD regimen and an anti-CD30 antibody conjugated to an auristatin compound provides synergistic therapeutic effects in the treatment of HL.

[0021] In one embodiment, methods for treating Hodgkin lymphoma in a subject are provided. The methods comprise administering to a subject in need thereof gemcitabine and an antibody-drug conjugate compound. Administration of the antibody-drug conjugate compound and gemcitabine provides a synergistic effect

in the treatment of Hodgkin lymphoma in the patient. The antibody-drug conjugate compound is an anti-CD30 antibody conjugated to an auristatin compound.

[0022] In another embodiment, methods for treating Hodgkin lymphoma in a subject consist essentially of administering to a subject in need thereof gemcitabine and an antibody-drug conjugate compound. Administration of the antibody-drug conjugate compound and gemcitabine provides a synergistic effect in the treatment of Hodgkin lymphoma in the patient. The antibody-drug conjugate compound is an anti-CD30 antibody conjugated to an auristatin compound.

[0023] The antibody-drug conjugate compound is typically delivered over a treatment cycle. The treatment cycle can be any suitable length of time. In one aspect, it is three or four weeks.

[0024] Also provided by the present invention is the use of an antibody-drug conjugate compound in the manufacture of a medicament to be administered in combination therapy with gemcitabine for the treatment of Hodgkin lymphoma in a subject. Administration of the antibody-drug conjugate compound and gemcitabine provides a synergistic effect in the treatment of Hodgkin lymphoma in the patient. The antibody-drug conjugate compound is an anti-CD30 antibody conjugated to an auristatin compound.

[0025] In one embodiment, methods of the present invention comprise administering to a subject in need thereof a chemotherapeutic regimen comprising doxorubicin, bleomycin, vinblastine and dacarbazine (ABVD) and an antibody-drug conjugate compound. Administration of the antibody-drug conjugate compound and the chemotherapeutic regimen comprising doxorubicin, bleomycin, vinblastine, and dacarbazine provides a synergistic effect in the treatment of Hodgkin lymphoma in the patient. The antibody-drug conjugate compound is an anti-CD30 antibody conjugated to an auristatin compound.

[0026] In another embodiment, the methods for treating Hodgkin lymphoma in a subject consist essentially of administering to a subject in need thereof a chemotherapeutic regimen comprising doxorubicin, bleomycin, vinblastine and dacarbazine and an antibody-drug conjugate compound. Administration of the antibody-drug conjugate compound and the chemotherapeutic regimen comprising doxorubicin, bleomycin, vinblastine and dacarbazine provides a synergistic effect in the treatment of Hodgkin lymphoma in the patient. The antibody-drug conjugate compound is an anti-CD30 antibody conjugated to an auristatin compound.

[0027] The antibody-drug conjugate compound is typically delivered over a treatment cycle. The treatment cycle can be any suitable length of time. In one aspect, it is three or four weeks.

[0028] Also provided by the present invention is the use of an antibody-drug conjugate compound in the manufacture of a medicament to be administered in combination therapy with the chemotherapeutic regimen comprising doxorubicin, bleomycin, vinblastine, and dacarbazine for the treatment of Hodgkin lymphoma in a subject. Administration of the antibody-drug conjugate compound and the chemotherapeutic regimen provides a synergistic effect in the treatment of Hodgkin lymphoma in the patient. The antibody-drug conjugate compound is an anti-CD30 antibody conjugated to an auristatin compound.

C. Definitions and Abbreviations

[0029] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art pertinent to the methods and compositions described. As used herein, the following terms and phrases have the meanings ascribed to them unless specified otherwise.

[0030] The term “inhibit” or “inhibition of” as used herein means to a reduce by a measurable amount, or to prevent entirely.

[0031] The transitional phrase "consisting essentially of" as used herein limits the scope of a claim to the specified active agents or steps and those additional active agents and steps that do not materially affect the properties of the specificed active agents.

[0032] The term “agent” as used herein means an element, compound, or molecular entity, including, *e.g.*, a pharmaceutical, therapeutic, or pharmacologic compound. Agents can be natural or synthetic or a combination thereof. A “therapeutic anti-cancer agent” is an agent that exerts a therapeutic (*e.g.*, beneficial) effect on cancer cells either alone or in combination with another agent. Typically, therapeutic anti-cancer agents useful in accordance with the methods and compositions described herein are those that exert a cytotoxic and/or cytostatic effect on target cells.

[0033] “Cytotoxic effect,” in reference to the effect of an agent on a cell, means killing of the cell. “Cytostatic effect” means an inhibition of cell proliferation.

A "cytotoxic agent" means an agent that has a cytotoxic or cytostatic effect on a cell, thereby depleting or inhibiting the growth of, respectively, cells within a cell population.

[0034] The term "deplete," in the context of the effect of an anti- CD30-antibody-drug conjugate on CD30-expressing cells, refers to a reduction or elimination of the CD30-expressing cells.

[0035] The terms "specific binding" and "specifically binds" mean that the anti-CD30 antibody will react, in a highly selective manner, with its corresponding target, CD30 and not with the multitude of other antigens. Typically, the anti-CD30 antibody binds with an affinity of at least about $1x10^{-7}$ M, and preferably 10^{-8} M to 10^{-9} M, 10^{-10} M, 10^{-11} M, or 10^{-12} M.

[0036] The term "antibody" as used herein refers to (a) immunoglobulin polypeptides and immunologically active portions of immunoglobulin polypeptides, *i.e.*, polypeptides of the immunoglobulin family, or fragments thereof, that contain an antigen binding site that immunospecifically binds to a specific antigen (*e.g.*, CD30), or (b) conservatively substituted derivatives of such immunoglobulin polypeptides or fragments that immunospecifically bind to the antigen (*e.g.*, CD30). Antibodies are generally described in, for example, Harlow & Lane, *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 1988). As used herein, the term "antibody" includes antibodies that have been modified by covalent attachment of a heterologous molecule such as, *e.g.*, by attachment of a heterologous polypeptide, or by glycosylation, acetylation or phosphorylation not normally associated with the antibody, and the like.

[0037] The term "monoclonal antibody" refers to an antibody that is derived from a single cell clone, including any eukaryotic or prokaryotic cell clone, or a phage clone, and not the method by which it is produced. Thus, the term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology.

[0038] The terms "identical" or "percent identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence. To determine the percent identity, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first

amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % identity = # of identical positions/total # of positions (*e.g.*, overlapping positions) x 100). In certain embodiments, the two sequences are the same length.

[0039] The term "substantially identical," in the context of two nucleic acids or polypeptides, refers to two or more sequences or subsequences that have at least 70% or at least 75% identity; more typically at least 80% or at least 85% identity; and even more typically at least 90%, at least 95%, or at least 98% identity (as determined using one of the methods set forth).

[0040] "Similarity" or "percent similarity" in the context of two or more polypeptide sequences, refer to two or more sequences or subsequences that have a specified percentage of amino acid residues that are the same or conservatively substituted when compared and aligned for maximum correspondence, as measured using one of the methods set forth *infra*. By way of example, a first amino acid sequence can be considered similar to a second amino acid sequence when the first amino acid sequence is at least 50%, 60%, 70%, 75%, 80%, 90%, or even 95% identical, or conservatively substituted, to the second amino acid sequence when compared to an equal number of amino acids as the number contained in the first sequence, or when compared to an alignment of polypeptides that has been aligned by a computer similarity program known in the art (*see infra*).

[0041] The terms "substantial similarity" or "substantial similarity," in the context of polypeptide sequences, indicates that a polypeptide region has a sequence with at least 70%, typically at least 80%, more typically at least 85%, and even more typically at least 90% or at least 95% sequence similarity to a reference sequence. For example, a polypeptide is substantially similar to a second polypeptide, for example, where the two peptides differ by one or more conservative substitutions.

[0042] The determination of percent identity or percent similarity between two sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two

sequences is the algorithm of Karlin and Altschul, 1990, *Proc. Natl. Acad. Sci. USA* 87:2264-2268, modified as in Karlin and Altschul, 1993, *Proc. Natl. Acad. Sci. USA* 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, *et al.*, 1990, *J. Mol. Biol.* 215:403-410. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to a nucleic acid encoding a protein of interest. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to protein of interest. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, 1997, *Nucleic Acids Res.* 25:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules (Id.). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. (*See*, *e.g.*, Internet web site address: www.ncbi.nlm.nih.gov.) Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS (1989). Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. Additional algorithms for sequence analysis are known in the art and include ADVANCE and ADAM as described in Torellis and Robotti, 1994, *Comput. Appl. Biosci.* 10:3-5; and FASTA described in Pearson and Lipman, 1988, *Proc. Natl. Acad. Sci.* 85:2444-8. Within FASTA, ktup is a control option that sets the sensitivity and speed of the search. If ktup=2, similar regions in the two sequences being compared are found by looking at pairs of aligned residues; if ktup=1, single aligned amino acids are examined. ktup can be set to 2 or 1 for protein sequences, or from 1 to 6 for DNA sequences. The default if ktup is not specified is 2 for proteins and 6 for DNA. For a further description of FASTA parameters, *see*, *e.g.*, bioweb.pasteur.fr/docs/man/man/fasta.1.html#sect2, the contents of which are incorporated herein by reference.

[0043] Alternatively, protein sequence alignment may be carried out using the CLUSTAL W algorithm, as described by Higgins *et al.*, 1996, *Methods Enzymol.* 266:383-402.

[0044] As used herein, the terms "treatment" or "treat" refer to slowing, stopping, or reversing the progression of HL in a subject, as evidenced by a decrease or elimination of a clinical or diagnostic symptom of the disease. Treatment can include, for example, a decrease in the severity of a symptom, the number of symptoms, or frequency of relapse, *e.g.,* the inhibition of tumor growth, the arrest of tumor growth, or the regression of already existing tumors.

[0045] The term "anti-cancer agent" as used herein, refers to any agent that slows, stops, or reverses the progression of cancer in a subject. For example, an anti-cancer agent is an agent that inhibits tumor growth, arrests tumor growth, and/or causes the regression of already existing tumors. Anti-inflammatory agents or other agents administered to a subject with cancer to treat symptoms associated with cancer, including, for example inflammation, weight loss, and general malaise are not considered anti-cancer agents.

[0046] The term "pharmaceutically acceptable" as used herein refers to those compounds, materials, compositions, and/or dosage forms that are, within the scope of sound medical judgment, suitable for contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problems or complications commensurate with a reasonable benefit/risk ratio. The term "pharmaceutically compatible ingredient" refers to a pharmaceutically acceptable diluent, adjuvant, excipient, or vehicle with which an antibody-drug conjugate compound is administered.

[0047] The term "therapeutically effective amount" as used herein to refer to combination therapy means the amount of the combination of agents taken together so that the combined effect elicits the desired biological or medicinal response, i.e., inhibits the occurrence or ameliorate one or more clinical or diagnostic symptoms of Hodgkin lymphoma. For example, the "therapeutically effective amount" as used herein to refer to combination therapy would be the amount of the antibody-drug conjugate compound and the amount of the chemotherapeutic drug(s) that when administered together, either sequentially or simultaneously, on the same or different days during a treatment cycle, have a combined effect that is therapeutically effective and synergistic. Further, it will be recognized by one skilled in the art that in the case of combination therapy with a therapeutically effective amount, as in the example above, the amount of the antibody-drug conjugate compound and/or the amount of the chemotherapeutic drug(s) individually may or may not be therapeutically effective.

[0048] The abbreviation "MMAE" refers to monomethyl auristatin E.

[0049] The abbreviation "MMAF" refers to dovaline-valine-dolaisoleunine-dolaproine-phenylalanine.

[0050] The abbreviations "fk" and "phe-lys" refer to the dipeptide phenylalanine-lysine.

[0051] The abbreviations "vc" and "val-cit" refer to the dipeptide valine-citrulline.

[0052] The term "compound" refers to and encompasses the chemical compound itself as well as, whether explicitly stated or not, and unless the context makes clear that the following are to be excluded: amorphous and crystalline forms of the compound, including polymorphic forms, where these forms may be part of a mixture or in isolation; free acid and free base forms of the compound, which are typically the forms shown in the structures provided herein; isomers of the compound, which refers to optical isomers, and tautomeric isomers, where optical isomers include enantiomers and diastereomers, chiral isomers and non-chiral isomers, and the optical isomers include isolated optical isomers as well as mixtures of optical isomers including racemic and non-racemic mixtures; where an isomer may be in isolated form or in a mixture with one or more other isomers; isotopes of the compound, including deuterium- and tritium-containing compounds, and including compounds containing radioisotopes, including therapeutically- and diagnostically-effective radioisotopes; multimeric forms of the compound, including dimeric, trimeric, etc. forms; salts of the compound, preferably pharmaceutically acceptable salts, including acid addition salts and base addition salts, including salts having organic counterions and inorganic counterions, and including zwitterionic forms, where if a compound is associated with two or more counterions, the two or more counterions may be the same or different; and solvates of the compound, including hemisolvates, monosolvates, disolvates, etc., including organic solvates and inorganic solvates, said inorganic solvates including hydrates; where if a compound is associated with two or more solvent molecules, the two or more solvent molecules may be the same or different. In some instances, reference made herein to a compound of the invention will include an explicit reference to one or of the above forms, *e.g.*, salts and/or solvates, however, this reference is for emphasis only, and is not to be construed as excluding other of the above forms as identified above.

[0053] As used herein, "pharmaceutically acceptable salts" refer to derivatives of the disclosed compounds wherein the parent compound is modified by making acid or base salts thereof. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. The pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, and the like. These physiologically acceptable salts are prepared by methods known in the art, *e.g.*, by dissolving the free amine bases with an excess of the acid in aqueous alcohol, or neutralizing a free carboxylic acid with an alkali metal base such as a hydroxide, or with an amine

[0054] Unless otherwise noted, the term "alkyl" refers to a saturated straight or branched hydrocarbon having from about 1 to about 20 carbon atoms (and all combinations and subcombinations of ranges and specific numbers of carbon atoms therein), with from about 1 to about 8 carbon atoms being preferred. Examples of alkyl groups are methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *iso*-butyl, *sec*-butyl, *tert*-butyl, *n*-pentyl, 2-pentyl, 3-pentyl, 2-methyl-2-butyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, *n*-decyl, 3-methyl-2-butyl, 3-methyl-1-butyl, 2-methyl-1-butyl, 1-hexyl, 2-hexyl, 3-hexyl, 2-methyl-2-pentyl, 3-methyl-2-pentyl, 4-methyl-2-pentyl, 3-methyl-3-pentyl, 2-methyl-3-pentyl, 2,3-dimethyl-2-butyl, and 3,3-dimethyl-2-butyl.

[0055] Alkyl groups, whether alone or as part of another group, can be optionally substituted with one or more groups, preferably 1 to 3 groups (and any additional substituents selected from halogen), including, but not limited to, -halogen, -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -$C(O)NH_2$, -C(O)NHR', -$C(O)N(R')_2$, -NHC(O)R', -SR', -SO_3R', -$S(O)_2R'$, -S(O)R', -OH, =O, -N_3, -NH_2, -NH(R'), -$N(R')_2$ and -CN, where each R' is independently selected from -H, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, or -aryl, and wherein said -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl),

-aryl, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, and -C_2-C_8 alkynyl groups can be optionally further substituted with one or more groups including, but not limited to, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, -halogen, -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -aryl, -C(O)R'', -OC(O)R'', -C(O)OR'', -C(O)NH_2 , -C(O)NHR'', -C(O)N(R'')$_2$, -NHC(O)R'', -SR'', -SO_3R'', -S(O)$_2$R'', -S(O)R'', -OH, -N_3 , -NH_2, -NH(R''), -N(R'')$_2$ and -CN, where each R'' is independently selected from -H, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, or -aryl.

[0056] Unless otherwise noted, the terms "alkenyl" and "alkynyl" refer to straight and branched carbon chains having from about 2 to about 20 carbon atoms (and all combinations and subcombinations of ranges and specific numbers of carbon atoms therein), with from about 2 to about 8 carbon atoms being preferred. An alkenyl chain has at least one double bond in the chain and an alkynyl chain has at least one triple bond in the chain. Examples of alkenyl groups include, but are not limited to, ethylene or vinyl, allyl, -1-butenyl, -2-butenyl, -isobutylenyl, -1-pentenyl, -2-pentenyl, -3-methyl-1-butenyl, -2-methyl-2-butenyl, and -2,3-dimethyl-2-butenyl. Examples of alkynyl groups include, but are not limited to, acetylenic, propargyl, acetylenyl, propynyl, -1-butynyl, -2-butynyl, -1-pentynyl, -2-pentynyl, and -3-methyl-1 butynyl.

[0057] Alkenyl and alkynyl groups, whether alone or as part of another group, can be optionally substituted with one or more groups, preferably 1 to 3 groups (and any additional substituents selected from halogen), including but not limited to, -halogen, -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2 , -C(O)NHR', -C(O)N(R')$_2$, -NHC(O)R', -SR', -SO_3R', -S(O)$_2$R', -S(O)R', -OH, =O, -N_3 , -NH_2, -NH(R'), -N(R')$_2$ and -CN, where each R' is independently selected from -H, -C_1-C_8 alkyl, -C_2-C_8 alkyenl, -C_2-C_8 alkynyl, or -aryl and wherein said -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -aryl, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, and -C_2-C_8 alkynyl groups can be optionally further substituted with one or more substituents including, but not limited to, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, -halogen, -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2C_8 alkynyl), -aryl, -C(O)R'', -OC(O)R'', -C(O)OR'', -C(O)NH_2 , -C(O)NHR'', -C(O)N(R'')$_2$, -NHC(O)R'', -SR'', -SO_3R'', -S(O)$_2$R'', -S(O)R'', -OH, -N_3 , -NH_2, -NH(R''), -N(R'')$_2$ and -CN, where each R'' is independently selected from -H, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, or -aryl.

[0058] Unless otherwise noted, the term "alkylene" refers to a saturated branched or straight chain hydrocarbon radical having from about 1 to about 20 carbon atoms (and all combinations and subcombinations of ranges and specific numbers of carbon atoms therein), with from about 1 to about 8 carbon atoms being preferred and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkane. Typical alkylenes include, but are not limited to, methylene, ethylene, propylene, butylene, pentylene, hexylene, heptylene, ocytylene, nonylene, decalene, 1,4-cyclohexylene, and the like. Alkylene groups, whether alone or as part of another group, can be optionally substituted with one or more groups, preferably 1 to 3 groups (and any additional substituents selected from halogen), including, but not limited to, -halogen, -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2, -C(O)NHR', -C(O)N(R')$_2$, -NHC(O)R', -SR', -SO_3R', -$S(O)_2$R', -S(O)R', -OH, =O, -N_3, -NH_2, -NH(R'), -N(R')$_2$ and -CN, where each R' is independently selected from -H, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, or -aryl and wherein said -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -aryl, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, and -C_2-C_8 alkynyl groups can be further optionally substituted with one or more substituents including, but not limited to, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, -halogen, -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -aryl, -C(O)R'', -OC(O)R'', -C(O)OR'', -C(O)NH_2, -C(O)NHR'', -C(O)N(R'')$_2$, -NHC(O)R'', -SR'', -SO_3R'', -$S(O)_2$R'', -S(O)R'', -OH, -N_3, -NH_2, -NH(R''), -N(R'')$_2$ and -CN, where each R'' is independently selected from -H, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, or -aryl.

[0059] Unless otherwise noted, the term "alkenylene" refers to an optionally substituted alkylene group containing at least one carbon-carbon double bond. Exemplary alkenylene groups include, for example, ethenylene (-CH=CH-) and propenylene (-CH=CHCH_2-).

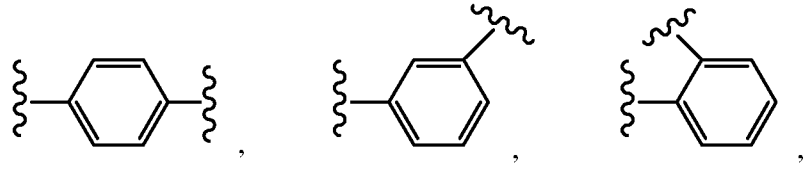
[0060] Unless otherwise noted, the term "alkynylene" refers to an optionally substituted alkylene group containing at least one carbon-carbon triple bond. Exemplary alkynylene groups include, for example, acetylene (-C≡C-), propargyl (-CH_2C≡C-), and 4-pentynyl (-CH_2CH_2CH_2C≡CH-).

[0061] Unless otherwise noted, the term "aryl" refers to a monovalent aromatic hydrocarbon radical of 6-20 carbon atoms (and all combinations and

subcombinations of ranges and specific numbers of carbon atoms therein) derived by the removal of one hydrogen atom from a single carbon atom of a parent aromatic ring system. Some aryl groups are represented in the exemplary structures as "Ar". Typical aryl groups include, but are not limited to, radicals derived from benzene, substituted benzene, phenyl, naphthalene, anthracene, biphenyl, and the like.

[0062] An aryl group, whether alone or as part of another group, can be optionally substituted with one or more, preferably 1 to 5, or even 1 to 2 groups including, but not limited to, -halogen, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2, -C(O)NHR', -C(O)N(R')$_2$, -NHC(O)R', -SR', -SO_3R', -S(O)$_2$R', -S(O)R', -OH, -NO_2, -N_3, -NH_2, -NH(R'), -N(R')$_2$ and -CN, where each R' is independently selected from -H, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, or -aryl and wherein said -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), and -aryl groups can be further optionally substituted with one or more substituents including, but not limited to, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, -halogen, -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -aryl, -C(O)R'', -OC(O)R'', -C(O)OR'', -C(O)NH_2, -C(O)NHR'', -C(O)N(R'')$_2$, -NHC(O)R'', -SR'', -SO_3R'', -S(O)$_2$R'', -S(O)R'', -OH, -N_3, -NH_2, -NH(R''), -N(R'')$_2$ and -CN, where each R'' is independently selected from -H, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, or -aryl.

[0063] Unless otherwise noted, the term "arylene" refers to an optionally substituted aryl group which is divalent (*i.e.,* derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent aromatic ring system) and can be in the ortho, meta, or para configurations as shown in the following structures with phenyl as the exemplary aryl group:



Typical "-(C_1-C_8 alkylene)aryl," "-(C_2-C_8 alkenylene)aryl", "and -(C_2-C_8 alkynylene)aryl" groups include, but are not limited to, benzyl, 2-phenylethan-1-yl, 2-phenylethen-1-yl, naphthylmethyl, 2-naphthylethan-1-yl, 2-naphthylethen-1-yl, naphthobenzyl, 2-naphthophenylethan-1-yl and the like.

[0064] Unless otherwise noted, the term "heterocycle," refers to a monocyclic, bicyclic, or polycyclic ring system having from 3 to 14 ring atoms (also referred to as ring members) wherein at least one ring atom in at least one ring is a heteroatom selected from N, O, P, or S (and all combinations and subcombinations of ranges and specific numbers of carbon atoms and heteroatoms therein). The heterocycle can have from 1 to 4 ring heteroatoms independently selected from N, O, P, or S. One or more N, C, or S atoms in a heterocycle can be oxidized. A monocylic heterocycle preferably has 3 to 7 ring members (e.g., 2 to 6 carbon atoms and 1 to 3 heteroatoms independently selected from N, O, P, or S), and a bicyclic heterocycle preferably has 5 to 10 ring members (*e.g.,* 4 to 9 carbon atoms and 1 to 3 heteroatoms independently selected from N, O, P, or S). The ring that includes the heteroatom can be aromatic or non-aromatic. Unless otherwise noted, the heterocycle is attached to its pendant group at any heteroatom or carbon atom that results in a stable structure.

[0065] Heterocycles are described in Paquette, "Principles of Modern Heterocyclic Chemistry" (W.A. Benjamin, New York, 1968), particularly Chapters 1, 3, 4, 6, 7, and 9; "The Chemistry of Heterocyclic Compounds, A series of Monographs" (John Wiley & Sons, New York, 1950 to present), in particular Volumes 13, 14, 16, 19, and 28; and *J. Am. Chem. Soc.* 82:5566 (1960).

[0066] Unless otherwise noted, the term “heterocyclo” refers to an optionally substituted heterocycle group as defined above that is divalent (*i.e.,* derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent heterocyclic ring system).

[0067] Examples of “heterocycle” groups include by way of example and not limitation pyridyl, dihydropyridyl, tetrahydropyridyl (piperidyl), thiazolyl, pyrimidinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, imidazolyl, tetrazolyl, benzofuranyl, thianaphthalenyl, indolyl, indolenyl, quinolinyl, isoquinolinyl, benzimidazolyl, piperidinyl, 4-piperidonyl, pyrrolidinyl, 2-pyrrolidonyl, pyrrolinyl, tetrahydrofuranyl, bis-tetrahydrofuranyl, tetrahydropyranyl, bis-tetrahydropyranyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl, octahydroisoquinolinyl, azocinyl, triazinyl, 6H-1,2,5-thiadiazinyl, 2H,6H-1,5,2-dithiazinyl, thienyl, thianthrenyl, pyranyl, isobenzofuranyl, chromenyl, xanthenyl, phenoxathinyl, 2H-pyrrolyl, isothiazolyl, isoxazolyl, pyrazinyl, pyridazinyl, indolizinyl, isoindolyl, 3H-indolyl, 1H-indazolyl, purinyl, 4H-quinolizinyl, phthalazinyl, naphthyridinyl, quinoxalinyl, quinazolinyl, cinnolinyl, pteridinyl, 4H-carbazolyl, carbazolyl, β-carbolinyl,

phenanthridinyl, acridinyl, pyrimidinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, furazanyl, phenoxazinyl, isochromanyl, chromanyl, imidazolidinyl, imidazolinyl, pyrazolidinyl, pyrazolinyl, piperazinyl, indolinyl, isoindolinyl, quinuclidinyl, morpholinyl, oxazolidinyl, benzotriazolyl, benzisoxazolyl, oxindolyl, benzoxazolinyl, and isatinoyl. Preferred "heterocycle" groups include, but are not limited to, benzofuranyl, benzothiophenyl, indolyl, benzopyrazolyl, coumarinyl, isoquinolinyl, pyrrolyl, thiophenyl, furanyl, thiazolyl, imidazolyl, pyrazolyl, triazolyl, quinolinyl, pyrimidinyl, pyridinyl, pyridonyl, pyrazinyl, pyridazinyl, isothiazolyl, isoxazolyl and tetrazolyl.

[0068] A heterocycle group, whether alone or as part of another group, can be optionally substituted with one or more groups, preferably 1 to 2 groups, including but not limited to, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, -halogen, -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2 , -C(O)NHR', -C(O)N(R')$_2$, -NHC(O)R', -SR', -SO_3R', -$S(O)_2$R', -S(O)R', -OH, -N_3 , -NH_2, -NH(R'), -N(R')$_2$ and -CN, where each R' is independently selected from -H, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, or -aryl and wherein said -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, and -aryl groups can be further optionally substituted with one or more substituents including, but not limited to, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, -halogen, -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -aryl, -C(O)R'', -OC(O)R'', -C(O)OR'', -C(O)NH_2 , -C(O)NHR'', -C(O)N(R'')$_2$, -NHC(O)R'', -SR'', -SO_3R'', -$S(O)_2$R'', -S(O)R'', -OH, -N_3 , -NH_2, -NH(R''), -N(R'')$_2$ and -CN, where each R'' is independently selected from -H, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, or aryl.

[0069] By way of example and not limitation, carbon-bonded heterocycles can be bonded at the following positions: position 2, 3, 4, 5, or 6 of a pyridine; position 3, 4, 5, or 6 of a pyridazine; position 2, 4, 5, or 6 of a pyrimidine; position 2, 3, 5, or 6 of a pyrazine; position 2, 3, 4, or 5 of a furan, tetrahydrofuran, thiofuran, thiophene, pyrrole or tetrahydropyrrole; position 2, 4, or 5 of an oxazole, imidazole or thiazole; position 3, 4, or 5 of an isoxazole, pyrazole, or isothiazole; position 2 or 3 of an aziridine; position 2, 3, or 4 of an azetidine; position 2, 3, 4, 5, 6, 7, or 8 of a quinoline; or position 1, 3, 4, 5, 6, 7, or 8 of an isoquinoline. Still more typically, carbon bonded heterocycles include 2-pyridyl, 3-pyridyl, 4-pyridyl, 5-pyridyl, 6-pyridyl, 3-pyridazinyl, 4-pyridazinyl, 5-pyridazinyl, 6-pyridazinyl, 2-pyrimidinyl, 4-

pyrimidinyl, 5-pyrimidinyl, 6-pyrimidinyl, 2-pyrazinyl, 3-pyrazinyl, 5-pyrazinyl, 6-pyrazinyl, 2-thiazolyl, 4-thiazolyl, or 5-thiazolyl.

[0070] By way of example and not limitation, nitrogen bonded heterocycles can be bonded at position 1 of an aziridine, azetidine, pyrrole, pyrrolidine, 2-pyrroline, 3-pyrroline, imidazole, imidazolidine, 2-imidazoline, 3-imidazoline, pyrazole, pyrazoline, 2-pyrazoline, 3-pyrazoline, piperidine, piperazine, indole, indoline, or 1H-indazole; position 2 of a isoindole, or isoindoline; position 4 of a morpholine; and position 9 of a carbazole, or β-carboline. Still more typically, nitrogen bonded heterocycles include 1-aziridyl, 1-azetedyl, 1-pyrrolyl, 1-imidazolyl, 1-pyrazolyl, and 1-piperidinyl.

[0071] Unless otherwise noted, the term "carbocycle," refers to a saturated or unsaturated non-aromatic monocyclic, bicyclic, or polycyclic ring system having from 3 to 14 ring atoms (and all combinations and subcombinations of ranges and specific numbers of carbon atoms therein) wherein all of the ring atoms are carbon atoms. Monocyclic carbocycles preferably have 3 to 6 ring atoms, still more preferably 5 or 6 ring atoms. Bicyclic carbocycles preferably have 7 to 12 ring atoms, *e.g.*, arranged as a bicyclo [4,5], [5,5], [5,6] or [6,6] system, or 9 or 10 ring atoms arranged as a bicyclo [5,6] or [6,6] system. The term "carbocycle" includes, for example, a monocyclic carbocycle ring fused to an aryl ring (*e.g.*, a monocyclic carbocycle ring fused to a benzene ring). Carbocyles preferably have 3 to 8 carbon ring atoms.

[0072] Carbocycle groups, whether alone or as part of another group, can be optionally substituted with, for example, one or more groups, preferably 1 or 2 groups (and any additional substituents selected from halogen), including, but not limited to, -halogen, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2, -C(O)NHR', -C(O)N(R')$_2$, -NHC(O)R', -SR', -SO_3R', -$S(O)_2$R', -S(O)R', -OH, =O, -N_3, -NH_2, -NH(R'), -N(R')$_2$ and -CN, where each R' is independently selected from -H, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, or -aryl and wherein said -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), and -aryl groups can be further optionally substituted with one or more substituents including, but not limited to, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, -halogen, -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -aryl, -C(O)R'', -OC(O)R'', -C(O)OR'', -C(O)NH_2, -C(O)NHR'', -C(O)N(R'')$_2$, -NHC(O)R'', -SR'', -SO_3R'', -$S(O)_2$R'', -S(O)R'', -OH, -N_3, -NH_2, -NH(R''),

-$N(R'')_2$ and -CN, where each R'' is independently selected from -H, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, or -aryl.

[0073] Examples of monocyclic carbocylic substituents include -cyclopropyl, -cyclobutyl, -cyclopentyl, -1-cyclopent-1-enyl, -1-cyclopent-2-enyl, -1-cyclopent-3-enyl, cyclohexyl, -1-cyclohex-1-enyl, -1-cyclohex-2-enyl, -1-cyclohex-3-enyl, -cycloheptyl, -cyclooctyl. -1,3-cyclohexadienyl, -1,4-cyclohexadienyl, -1,3-cycloheptadienyl, -1,3,5-cycloheptatrienyl, and –cyclooctadienyl.

[0074] A "carbocyclo," whether used alone or as part of another group, refers to an optionally substituted carbocycle group as defined above that is divalent (i.e., derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent carbocyclic ring system).

[0075] When any variable occurs more than one time in any constituent or in any formula, its definition in each occurrence is independent of its definition at every other. Combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

[0076] Unless otherwise indicated by context, a hyphen (-) designates the point of attachment to the pendant molecule. Accordingly, the term "-(C_1-C_8 alkylene)aryl" or "-C_1-C_8 alkylene(aryl)" refers to a C_1-C_8 alkylene radical as defined herein wherein the alkylene radical is attached to the pendant molecule at any of the carbon atoms of the alkylene radical and one of the hydrogen atoms bonded to a carbon atom of the alkylene radical is replaced with an aryl radical as defined herein.

[0077] When a particular group is "substituted", that group may have one or more substituents, preferably from one to five substituents, more preferably from one to three substituents, most preferably from one to two substituents, independently selected from the list of substituents. The group can, however, generally have any number of substituents selected from halogen. Groups that are substituted are so indicated.

[0078] It is intended that the definition of any substituent or variable at a particular location in a molecule be independent of its definitions elsewhere in that molecule. It is understood that substituents and substitution patterns on the compounds of this invention can be selected by one of ordinary skill in the art to provide compounds that are chemically stable and that can be readily synthesized by techniques known in the art as well as those methods set forth herein.

[0079] Protective groups as used herein refer to groups which selectively block, either temporarily or permanently, one reactive site in a multifunctional compound. Suitable hydroxy-protecting groups for use in the present invention are pharmaceutically acceptable and may or may not need to be cleaved from the parent compound after administration to a subject in order for the compound to be active. Cleavage is through normal metabolic processes within the body. Hydroxy protecting groups are well known in the art, see, Protective Groups in Organic Synthesis by T. W. *Greene* and P. G. M. Wuts (John Wiley & sons, 3rd Edition) incorporated herein by reference in its entirety and for all purposes and include, for example, ether (e.g., alkyl ethers and silyl ethers including, for example, dialkylsilylether, trialkylsilylether, dialkylalkoxysilylether), ester, carbonate, carbamates, sulfonate, and phosphate protecting groups. Examples of hydroxy protecting groups include, but are not limited to, methyl ether; methoxymethyl ether, methylthiomethyl ether, (phenyldimethylsilyl)methoxymethyl ether, benzyloxymethyl ether, p-methoxybenzyloxymethyl ether, p-nitrobenzyloxymethyl ether, o-nitrobenzyloxymethyl ether, (4-methoxyphenoxy)methyl ether, guaiacolmethyl ether, t-butoxymethyl ether, 4-pentenyloxymethyl ether, siloxymethyl ether, 2-methoxyethoxymethyl ether, 2,2,2-trichloroethoxymethyl ether, bis(2-chloroethoxy)methyl ether, 2-(trimethylsilyl)ethoxymethyl ether, menthoxymethyl ether, tetrahydropyranyl ether, 1-methoxycylcohexyl ether, 4-methoxytetrahydrothiopyranyl ether, 4-methoxytetrahydrothiopyranyl ether S,S-Dioxide, 1-[(2-choro-4-methyl)phenyl]-4-methoxypiperidin-4-yl ether, 1-(2-fluorophneyl)-4-methoxypiperidin-4-yl ether, 1,4-dioxan-2-yl ether, tetrahydrofuranyl ether, tetrahydrothiofuranyl ether; substituted ethyl ethers such as 1-ethoxyethyl ether, 1-(2-chloroethoxy)ethyl ether, 1-[2-(trimethylsilyl)ethoxy]ethyl ether, 1-methyl-1-methoxyethyl ether, 1-methyl-1-benzyloxyethyl ether, 1-methyl-1-benzyloxy-2-fluoroethyl ether, 1-methyl-1phenoxyethyl ether, 2-trimethylsilyl ether, t-butyl ether, allyl ether, propargyl ethers, p-chlorophenyl ether, p-methoxyphenyl ether, benzyl ether, p-methoxybenzyl ether 3,4-dimethoxybenzyl ether, trimethylsilyl ether, triethylsilyl ether, tripropylsilylether, dimethylisopropylsilyl ether, diethylisopropylsilyl ether, dimethylhexylsilyl ether, t-butyldimethylsilyl ether, diphenylmethylsilyl ether, benzoylformate ester, acetate ester, chloroacetate ester, dichloroacetate ester, trichloroacetate ester, trifluoroacetate ester, methoxyacetate ester, triphneylmethoxyacetate ester, phenylacetate ester, benzoate ester, alkyl methyl

carbonate, alkyl 9-fluorenylmethyl carbonate, alkyl ethyl carbonate, alkyl 2,2,2,-trichloroethyl carbonate, 1,1,-dimethyl-2,2,2-trichloroethyl carbonate, alkylsulfonate, methanesulfonate, benzylsulfonate, tosylate, methylene acetal, ethylidene acetal, and t-butylmethylidene ketal. Preferred protecting groups are represented by the formulas $-R^a$, $-Si(R^a)(R^a)(R^a)$, $-C(O)R^a$, $-C(O)OR^a$, $-C(O)NH(R^a)$, $-S(O)_2R^a$, $-S(O)_2OH$, $P(O)(OH)_2$, and $-P(O)(OH)OR^a$, wherein R^a is C_1-C_{20} alkyl, C_2-C_{20} alkenyl, C_2-C_{20} alkynyl, -C_1-C_{20} alkylene(carbocycle), -C_2-C_{20} alkenylene(carbocycle), -C_2-C_{20} alkynylene(carbocycle), -C_6-C_{10} aryl, -C_1-C_{20} alkylene(aryl), -C_2-C_{20} alkenylene(aryl), -C_2-C_{20} alkynylene(aryl), -C_1-C_{20} alkylene(heterocycle), -C_2-C_{20} alkenylene(heterocycle), or -C_2-C_{20} alkynylene(heterocycle) wherein said alkyl, alkenyl, alkynyl, alkylene, alkenylene, and alkynylene radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A1, said carbocycle radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A2, said aryl radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A3, and said heterocycle radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A4. A1, A2, A3, and A4 are as defined herein.

D. The Antibody-Drug Conjugate

[0080] The methods described herein encompass the use of an antibody-drug conjugate compound in combination therapy for the treatment of HL. The antibody-drug conjugate compound for use in the present invention comprises an anti-CD30 antibody, *i.e.,* an antibody that specifically binds to CD30, linked to a drug moiety. The drug moiety is of the auristatin type which have been shown to interfere with microtubule dynamics and nuclear and cellular division and have anticancer activity. Auristatins of the present invention bind to tubulin and exert a cytotoxic or cytostatic effect on a HL cell line, *e.g.,* L540cy cell line. In some embodiments of the present invention, the auristatin drug is conjugated to the anti-CD30 antibody via a linker that is cleavable under intracellular conditions, such that cleavage of the linker releases the auristatin compound from the antibody in the intracellular environment. In yet other

embodiments, the linker unit is not cleavable and the drug is released by antibody degradation.

[0081] There are a number of different assays that can be used for determining whether an auristatin or resultant antibody-drug conjugate exerts a cytostatic or cytotoxic effect on a HL cell line. In one example for determining whether an auristatin or resultant antibody-drug conjugate exerts a cytostatic or cytotoxic effect on a HL cell line, a thymidine incorporation assay is used. For example, HL cells at a density of 5,000 cells/well of a 96-well plated is cultured for a 72-hour period and exposed to 0.5 μCi of ^{3}H-thymidine during the final 8 hours of the 72-hour period, and the incorporation of ^{3}H-thymidine into cells of the culture is measured in the presence and absence of the auristatin or antibody drug conjugate. The auristatin or resultant antibody-drug conjugate has a cytostatic or cytotoxic effect on the HL cell line if the cells of the culture have reduced ^{3}H-thymidine incorporation compared to cells of the same cell line cultured under the same conditions but not contacted with the auristatin or antibody drug conjugate.

[0082] For determining cytotoxicity, necrosis or apoptosis (programmed cell death) can be measured. Necrosis is typically accompanied by increased permeability of the plasma membrane; swelling of the cell, and rupture of the plasma membrane. Apoptosis is typically characterized by membrane blebbing, condensation of cytoplasm, and the activation of endogenous endonucleases. Determination of any of these effects on HL cells indicates that an auristatin or antibody-drug conjugate is useful in the treatment or prevention of HL.

[0083] In another example, for determining whether an auristatin or resultant antibody-drug conjugate exerts a cytostatic or cytotoxic effect on a HL cell line, cell viability is measured by determining in a cell the uptake of a dye such as neutral red, trypan blue, or ALAMAR™ blue (*see, e.g.*, Page *et al.*, 1993, *Intl. J. of Oncology* 3:473-476). In such an assay, the cells are incubated in media containing the dye, the cells are washed, and the remaining dye, reflecting cellular uptake of the dye, is measured spectrophotometrically. The protein-binding dye sulforhodamine B (SRB) can also be used to measure cytoxicity (Skehan *et al.*, 1990, *J. Nat'l Cancer Inst.* 82:1107-12). Preferred antibody drug conjugates include those with an IC_{50} value (defined as the mAB concentration that gives 50% cell kill) of less than 1000 ng/ml, preferably less than 500 ng/ml, more preferably less than 100 ng/ml, even most

preferably less than 50 or even less than 10 ng/ml on a Hodgkin lymphoma cell line, e.g., L540cy cell line.

[0084] Methods for determining whether a compound binds tubulin are known in the art. See, for example, Muller et al., *Anal. Chem* 2006, 78, 4390-4397; Hamel et al., *Molecular Pharmacology,* 1995 47: 965-976; and Hamel et al., *The Journal of Biological Chemistry*, 1990 265:28, 17141-17149. For purposes of the present invention, the relative affinity of a compound to tubulin can be determined. Preferred auristatins of the present invention bind tubulin with an affinity ranging from 10 fold lower (weaker affinity) that the binding affinity of MMAE to tubulin to 10 fold, 20 fold or even 100 fold higher (tighter affinity) than the binding affinity of MMAE to tubulin.

E. Gemcitabine

[0085] Some methods of the present invention encompass administering the antibody-drug conjugate compound and gemcitabine for the treatment of Hodgkin lymphoma.

[0086] Gemcitabine, 4-amino-1-[(2R,4R,5R)-3,3-difluoro-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]pyrimidin-2-one, is currently marketed under the label GEMZAR™ by Eli Lilly and Company. Gemcitabine, an analog of cytarabine, is a pyrimidine antimetabolite that has been found to demonstrate a broad spectrum of activity in HL.

[0087] The present invention encompasses combination therapy with an antibody-drug conjugate compound, gemcitabine and optionally one or more additional agents, *e.g.,* anti-cancer agents, including chemotherapeutic agents. For example, one or more of vinorelbine, dexamethasone, cisplatin, and doxorubicin, including pegylated liposomal doxorubicin, can be administered as part of the combination therapy (*e.g.,* GVD regimen). In some embodiments, however, gemcitabine will be the only chemotherapeutic agent administered as part of the combination therapy with the antibody-drug conjugate compound. For example, in some embodiments, during one or more cycles of therapy, gemcitabine will be the only chemotherapeutic agent administered as part of the combination therapy with the antibody-drug conjugate compound. In some embodiments, gemcitabine will be the only anti-cancer agent administered as part of the combination therapy with the

antibody-drug conjugate compound. In certain embodiments, doxorubicin, or, more specifically, pegylated liposomal doxorubicin, will be specifically excluded from the combination therapy. In some embodiments, vinorelbine and doxorubicin, or vinorelbine and pegylated liposomal doxorubicin, will be excluded from the combination therapy.

F. Doxorubicin, bleomycin, vinblastine, and dacarbazine therapy

[0088] The methods of the present invention encompass administering an antibody-drug conjugate compound and a chemotherapeutic regimen of doxorubicin, bleomycin, vinblastine, and dacarbazin as combination therapy for the treatment of Hodgkin lymphoma. Currently doxorubicin, bleomycin, vinblastine, and dacarbazin are administered together in a chemotherapeutic regimen referred to as ABVD.

[0089] The ABVD chemotherapy regimen is currently considered the standard of care in the 1st line treatment of HL. The ABVD chemotherapeutic regimen is typically administered to patients every two weeks in a four week treatment cycle. Typically, at day 1 and 15 of the four week interval, patients are treated with 25 mg/m^2 doxorubicin, 10 U/m^2 bleomycin, 6 mg/m^2 vinblastine, and 375 mg/m^2 dacarbazine.

[0090] The present invention encompasses combination therapy with an antibody-drug conjugate compound, doxorubicin, bleomycin, vinblastine, and dacarbazin (e.g., ABVD regimen) and optionally one or more additional agents, *e.g.*, anti-cancer agents, including chemotherapeutic agents. In certain embodiments, however, doxorubicin, bleomycin, vinblastine, and dacarbazin will be the only chemotherapeutic agents administered as part of the combination therapy with the antibody-drug conjugate compound. For example, in some embodiments, during one or more cycles of therapy, the ABVD regimen will be the only chemotherapeutic regimen administered as part of the combination therapy with the antibody-drug conjugate compound. In some embodiments, the ABVD regimen will be the only anti-cancer regimen administered as part of the combination therapy with the antibody-drug conjugate compound. In certain embodiments, doxorubicin will be specifically excluded from the combination therapy and the chemotherapeutic agents will comprise bleomycin, vinblastine, and dacarbazin.

G. Synergism

[0091] In preferred embodiments of the present invention, therapy with the antibody-drug conjugate compound and the chemotherapeutic drug(s) provides a synergistic effect in the treatment of Hodgkin lymphoma in the patient As used herein, the term "synergy" or "synergistic effect" when used in connection with a description of the efficacy of a combination of agents, means any measured effect of the combination which is greater than the effect predicted from a sum of the effects of the individual agents. Accordingly, the present invention encompasses embodiments wherein subjects treated with both the antibody-drug conjugate compound and the chemotherapeutic drug(s) have significantly better treatment outcomes than subjects treated with only the antibody-drug conjugate compound or only the chemotherapeutic drug(s) given the same administration and dosage regimens. The present invention encompasses embodiments wherein subjects have better treatment outcomes than would be expected from the sum of effects of treatment with the antibody-drug conjugate compound alone and the chemotherapeutic regimen alone given the same administration and dosage regimens.

[0092] Methods of determining such synergy are known in the art. In one example, syngeneic (same gene line) tumors are harvested from donor animals, disaggregated, counted and then injected back into syngeneic (same strain) host mice. Anticancer combinations are typically then injected at some later time point(s), either by intraperitoneal, intravenous or administered by the oral routes, and tumor growth rates and/or survival are determined, compared to untreated controls and controls exposed only to one of the therapies. Growth rates are typically measured for tumors growing in the front flank of the animal, wherein perpendicular diameters of tumor width are translated into an estimate of total tumor mass or volume. The time to reach a predetermined mass (*e.g.,* time for tumor to triplicate or time for tumor to quadruple) is then compared to the time required for equal tumor growth in the control animals. If the time to reach the predetermined mass for the animal treated with the combination therapy is greater than the value obtained from adding the time to reach the predetermined mass for the animal treated with therapy "A" and the animal treated with therapy "B" (*i.e.,* each therapy alone), the combination therapy can be said to provide a synergistic effect. In another example, the time to reach the predetermined mass for the animal treated with the combination therapy might not be

greater than the value obtained from adding the time to reach the predetermined mass for the animal treated with therapy "A" and the animal treated with therapy "B"; however, another measured effect of the combination which is greater than that predicted from a sum of the effects of the individual agents is sufficient to identify/determine the combination therapy as synergistic. For example, if the number of durable responses for the animals treated with the combination therapy is greater than the sum of the number of durable responses in each treatment arm alone, the combined therapy provides a synergistic effect. A durable response (DR) is defined as the absence of palpable tumor in the animal.

H. Administration

[0093] The antibody-drug conjugate and gemcitabine or the antibody-drug conjugate and the ABVD regimen are administered in such a way that they provide a synergistic effect in the treatment of HL in a patient. Administration can be by any suitable means provided that the administration provides the desired therapeutic effect, i.e., synergism. In preferred embodiments, the antibody-drug conjugate compound and gemcitabine or the antibody-drug conjugate compound and the ABVD regimen are administered during the same cycle of therapy, *e.g.,* during one cycle of therapy, e.g., a three or four week time period, both the antibody-drug conjugate compound and the specified chemotherapeutic drug(s) are administered to the subject. In some embodiments of the present invention, administration of the antibody-drug conjugate compound will be at such a time that it sensitizes cancerous cells to treatment with gemcitabine or the ABVD regimen, *i.e.,* sequentially, *e.g.,* immediately prior to chemotherapeutic treatment, *e.g.,* less than 2 hours prior to chemotherapeutic treatment.

[0094] The dosage of the antibody-drug conjugate compound administered to a patient with HL will also depend on frequency of administration. The present invention contemplates antibody-drug conjugate compound delivery once during the treatment cycle or by a split delivery.

[0095] The present invention encompasses embodiments wherein the antibody-drug conjugate compound will be administered in a dose range of 0.1 mg/kg to 2.7 mg/kg of the subject's body weight per dose, 0.2 mg/kg to 1.8 mg/kg of the subject's body weight per dose, 0.2 mg/kg to 1.2 mg/kg of the subject's body weight

per dose, 0.4 mg/kg to 1 mg/kg of the subject's body weight per dose, 1.0 mg/kg to 1.5 mg/kg of the subject's body weight per dose, and 0.5 mg/kg to 1 mg/kg of the subject's body weight per dose. Other ranges are encompassed by the present invention as long as they produce the desired result.

[0096] The present invention encompasses treatment schedules wherein the total dosage of the antibody-drug conjugate compound, administered to a patient with HL will be, for example, 0.1 mg/kg to 5 mg/kg, 0.1 mg/kg to 4 mg/kg, 0.1 mg/kg to 3.2 mg/kg, or 0.1 mg/kg to 2.7 mg/kg of the subject's body weight over a treatment cycle, *e.g.,* a 3 or 4 week time period. In some embodiments, the total dosage of the antibody-drug conjugate compound administered to a patient with HL will be, for example about 0.6 mg/kg to about 5 mg/kg, about 0.6 mg/kg to about 4 mg/kg, about 0.6 mg/kg to about 3.2 mg/kg, about 0.6 mg/kg to about 2.7 mg/kg, or even about 1.5 mg/kg to about about 3 mg/kg over a treatment cycle, *e.g.,* a 3 or 4 week time period. In some embodiments, the dosage will be about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1.0 mg/kg, about 1.1 mg/kg, about 1.2 mg/kg, about 1.3 mg/kg, about 1.4 mg/kg, about 1.5 mg/kg, about 1.6 mg/kg, about 1.7 mg/kg, about 1.8 mg/kg, about 1.9 mg/kg, about 2 mg/kg, about 2.1 mg/kg, about 2.2 mg/kg, about 2.3 mg/kg, about 2.4 mg/kg, about 2.5 mg/kg, about 2.6 mg/kg, about 2.7 mg/kg, about 2.8 mg/kg, about 2.9 mg/kg, about 3 mg/kg, about 3.1 mg/kg, about 3.2 mg/kg, about 3.3 mg/kg, about 3.4 mg/kg, about 3.5 mg/kg, about 3.6 mg/kg, about 3.7 mg/kg, or about 3.8 mg/kg of the subject's body weight over the treatment cycle, *e.g.,* a 3 or 4 week time period. The present invention contemplates administration of the drug for one or more treatment cycles, for example, 1, 2, 3, 4, 5, 6, or more, treatment cycles. In some embodiments, there will be periods of rest between one or more of the treatment cycles. For example, in some embodiments, there will be a period of rest between the second and third treatment cycle but not the first and second treatment cycle. In another embodiment, there might be a period of rest between the first and second treatment cycle but not the second and third treatment cycle. Dosing schedules include, for example, administering the antibody drug conjugate compound once during a treatment schedule, e.g., on day 1 of a 21 day cycle, twice during a treatment cycle, *e.g.,* on days 1 and 15 of a 28 day cycle, and three times during a treatment cycle, *e.g.,* on days 1, 8 and 15 of a 28 day cycle. Other dosage schedules are encompassed by the present invention.

[0097] The present invention encompasses treatment schedules wherein the antibody-drug conjugate compound is administered once during a treatment cycle, *e.g.,* a 3 or 4 week time period. For example, in some embodiments, the antibody-drug conjugate will be administered on the third week of a 3 or 4 week treatment cycle, e.g., on day 21 of a three or four week cycle. In some embodiments, the antibody-drug conjugate will be administered on day 1 of a 3 or 4 week treatment cycle, or on any other day of a three or four week treatment cycle. In some such embodiments, the dosage of the antibody-drug conjugate compound administered to a patient with HL will typically be, for example, 0.1 mg/kg to 5 mg/kg of the subject's body weight over the treatment cycle, *e.g.,* a 3 or 4 week time period. More typically, the dosage will be 0.1 mg/kg to 4 mg/kg, 0.1 mg/kg to 3.2 mg/kg, 0.1 mg/kg to 2.7 mg/kg, 1 mg/kg to 2.7 mg/kg, 1.5 mg/kg to 2.7 mg/kg, or 1.5 mg/kg to 2 mg/kg of the subject's body weight over the treatment cycle, *e.g.,* a 3 or 4 week time period. In some embodiments, the total dosage of the antibody-drug conjugate compound administered to a patient with HL will be, for example about 0.6 mg/kg to about 5 mg/kg, about 0.6 mg/kg to about 4 mg/kg, about 0.6 mg/kg to about 3.2 mg/kg, about 0.6 mg/kg to about 2.7 mg/kg, or even about 1.5 mg/kg to about about 3 mg/kg over a treatment cycle, *e.g.,* a 3 or 4 week time period. In some embodiments, the dosage will be about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1.0 mg/kg, about 1.1 mg/kg, about 1.2 mg/kg, about 1.3 mg/kg, about 1.4 mg/kg, about 1.5 mg/kg, about 1.6 mg/kg, about 1.7 mg/kg, about 1.8 mg/kg, about 1.9 mg/kg, about 2 mg/kg, about 2.1 mg/kg, about 2.2 mg/kg, about 2.3 mg/kg, about 2.4 mg/kg, about 2.5 mg/kg, about 2.6 mg/kg, about 2.7 mg/kg, about 2.8 mg/kg, about 2.9 mg/kg, about 3 mg/kg, about 3.1 mg/kg, about 3.2 mg/kg, about 3.3 mg/kg, about 3.4 mg/kg, about 3.5 mg/kg, about 3.6 mg/kg, about 3.7 mg/kg, or about 3.8 mg/kg of the subject's body weight over the treatment cycle.

[0098] In other embodiments the antibody-drug conjugate compound will be administered more than once during a treatment cycle. For example, in some embodiments, the antibody-drug conjugate compound will be administered weekly for three consecutive weeks in a three or four week treatment cycle. For example, in some embodiments, the antibody-drug conjugate compound will be administered on days 1, 8, and 15 of each 28 day treatment cycle. In some such embodiments, the dosage of the antibody-drug conjugate compound administered to a patient with HL can be, for example, 0.1 mg/kg to 5 mg/kg, 0.1 mg/kg to 4 mg/kg, 0.1 mg/kg to 3.2

mg/kg, or 0.1 mg/kg to 2.7 mg/kg of the subject's body weight over the treatment cycle. In some embodiments, the total dosage of the antibody-drug conjugate compound administered to a patient with HL will be, for example about 0.6 mg/kg to about 5 mg/kg, about 0.6 mg/kg to about 4 mg/kg, about 0.6 mg/kg to about 3.2 mg/kg, about 0.6 mg/kg to about 2.7 mg/kg, or even about 1.5 mg/kg to about about 3 mg/kg over the treatment cycle. In some embodiments, the dosage will be about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1.0 mg/kg, about 1.1 mg/kg, about 1.2 mg/kg, about 1.3 mg/kg, about 1.4 mg/kg, about 1.5 mg/kg, about 1.6 mg/kg, about 1.7 mg/kg, about 1.8 mg/kg, about 1.9 mg/kg, about 2 mg/kg, about 2.1 mg/kg, about 2.2 mg/kg, about 2.3 mg/kg, about 2.4 mg/kg, about 2.5 mg/kg, about 2.6 mg/kg, about 2.7 mg/kg, about 2.8 mg/kg, about 2.9 mg/kg, about 3 mg/kg, about 3.1 mg/kg, about 3.2 mg/kg, about 3.3 mg/kg, about 3.4 mg/kg, about 3.5 mg/kg, about 3.6 mg/kg, about 3.7 mg/kg, about 3.8 mg/kg , about 3.9 mg/kg or about 4.0 mg/kg of the subject's body weight over the treatment cycle. In some embodiments, the dosage will generally be 0.1 to 5 mg/kg of the subject's body weight, 0.1 mg/kg to 3.2 mg/kg of the subject's body weight, even more typically, 0.1 mg/kg to 2.7 mg/kg, 0.2 mg/kg to 1.8 mg/kg, 0.2 mg/kg to 1.2 mg/kg, 0.2 mg/kg to 1 mg/kg, 0.4 mg/kg to 1 mg/kg, or 0.4 mg/k g to 0.8 mg/kg of the subject's body weight on days 1, 8, and 15 of each 28 day cycle. In some embodiments, the dosage will be about 0.2 mg/kg, about 0.3 mg/kg, about 0.4 mg/kg, about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1.0 mg/kg, about 1.1 mg/kg, about 1.2 mg/kg, about 1.3 mg/kg/ about 1.4 mg/kg, or about 1.5 mg/kg of the subject's body weight on days 1, 8, and 15 of each 28 day cycle.

[0099] In even other embodiments the antibody-drug conjugate compound will be administered every two weeks in a four week treatment cycle. For example, in some embodiments, the antibody-drug conjugate compound will be administered on days 1 and 15 of each 28 day treatment cycle. In some such embodiments, the dosage of the antibody-drug conjugate compound administered to a patient with HL can be, for example, 0.1 mg/kg to 5 mg/kg, 0.1 mg/kg to 4 mg/kg, 0.1 mg/kg to 3.2 mg/kg, or 0.1 mg/kg to 2.7 mg/kg of the subject's body weight over the treatment cycle. In some embodiments, the total dosage of the antibody-drug conjugate compound administered to a patient with HL will be, for example about 0.6 mg/kg to about 5 mg/kg, about 0.6 mg/kg to about 4 mg/kg, about 0.6 mg/kg to about 3.2 mg/kg, about 0.6 mg/kg to about 2.7 mg/kg, or even about 1.5 mg/kg to about about 3 mg/kg over

the treatment cycle. In some embodiments, the dosage will be about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1.0 mg/kg, about 1.1 mg/kg, about 1.2 mg/kg, about 1.3 mg/kg, about 1.4 mg/kg, about 1.5 mg/kg, about 1.6 mg/kg, about 1.7 mg/kg, about 1.8 mg/kg, about 1.9 mg/kg, about 2 mg/kg, about 2.1 mg/kg, about 2.2 mg/kg, about 2.3 mg/kg, about 2.4 mg/kg, about 2.5 mg/kg, about 2.6 mg/kg, about 2.7 mg/kg, about 2.8 mg/kg, about 2.9 mg/kg, about 3 mg/kg, about 3.1 mg/kg, about 3.2 mg/kg, about 3.3 mg/kg, about 3.4 mg/kg, about 3.5 mg/kg, about 3.6 mg/kg, about 3.7 mg/kg, or about 3.8 mg/kg of the subject's body weight over the treatment cycle. In some embodiments, the dosage of the antibody-drug conjugate compound will generally be 0.1 mg/kg to 5 mg/kg of the subject's body weight, 0.1 mg/kg to 3.2 mg/kg of the subject's body weight, more typically 0.1 mg/kg to 2.7 mg/kg, even more typically 0.2 mg/kg to 1.8 mg/kg, 0.2 mg/kg to 1.2 mg/kg, 0.2 mg/kg to 1.5 mg/kg, 1 mg/kg to 1.5 mg/kg, or 0.5 to 1.2 mg/kg, of the subject's body weight on days 1 and 15 of each 28 day cycle. In some embodiments, the dosage will be about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1.0 mg/kg, about 1.1 mg/kg, about 1.2 mg/kg, about 1.3 mg/kg, about 1.4 mg/kg, about 1.5 mg/kg, about 1.6 mg/kg, about 1.7 mg/kg, or about 1.8 mg/kg of the subject's body weight on days 1 and 15 of each 28 day cycle.

[0100] It will be readily apparent to those skilled in the art that other antibody-drug conjugate compound doses or frequencies of administration that provide the desired therapeutic effect are suitable for use in the present invention.

[0101] Administration of the antibody-drug conjugate compound and gemcitabine can be on the same or different days provided that administration provides the desired thereapeutic effect. The present invention encompasses, for example, embodiments wherein gemcitabine is administered weekly for three consecutive weeks in a four week treatment cycle, e.g., on days 1, 8 and 15 of a 28 day cycle. The present invention encompasses, for example, embodiments wherein gemcitabine is administered two times in a four week treatment cycle, e.g., on days 1 and 15 of a 28 day cycle. The present invention encompasses, for example, embodiments wherein gemcitabine is administered two times in a three week treatment cycle, e.g., on days 1 and 8 or day 1 and 15 of a 21 day cycle. In some embodiments of the present invention, administration of the antibody-drug conjugate compound and gemcitabine will be on the same days, *e.g.,* on days 1, 8, and 15 of a four week cycle or on days 1 and 15 of a four week cycle. In some embodiments of

the present invention, administration of the antibody-drug conjugate compound and gemcitabine will be on the same and/or different days, *e.g,* the antibody drug conjugate will be administered on day 1 of a 21 day cycle and gemcitabine will be administered on day 1 and 8 or day 1 and 15 of the 21 day cycle. In some embodiments, the antibody-drug conjugate compound and gemcitabine will be administered on the same days and gemcitabine will be administered following completion of administration of the antibody-drug conjugate, *e.g.,* gemcitabine will be administered less than 2 hours following administration of the antibody-drug conjugate, e.g., 30 minutes following administration of the antibody-drug conjugate. Alternative treatment schedules are encompassed by the present invention as long as they produce the desired result.

[0102] In some embodiments, gemcitabine will be administered at levels currently indicated in the art for the treatment of HL or at lower or higher levels than those currently indicated in the art for the treatment of HL provided that such dosage provides the desired therapeutic effect. Embodiments of the present invention include, for example, those wherein the gemcitabine regimen is administered at about the MTD, maximum tolerated dose. Embodiments of the present invention include those wherein gemcitabine is administered in a dose range of about 100 mg/m^2 to about 2000 mg/m^2, about 500 mg/m^2 to about 1500 mg/m^2, about 500 mg/m^2 to about 1250 mg/m^2, or about 750 mg/m^2 to about 1250 mg/m^2 at each administration. In particularly preferred embodiments, gemcitabine is administered in a dose range of about 750 mg/m^2 to about 1250 mg/m^2 at each administration, or about 1000 mg/m^2 to about 1250 mg/m^2 at each administration. For example, in some embodiments, gemcitabine will be administered in a dose range of 750 mg/m^2 to about 1250 mg/m^2 or about 1000 mg/m^2 to about 1250 mg/m^2 on days 1, 8, and 15 of a 28 day treatment cycle. In some embodiments, gemcitabine will be administered in a dose range of 750 mg/m^2 to about 1250 mg/m^2 or about 1000 mg/m^2 to about 1250 mg/m^2 on days 1 and 15 or days of a 28 day treatment cycle. In some embodiments, gemcitabine will be administered in a dose range of 750 mg/m^2 to about 1250 mg/m^2 or about 1000 mg/m^2 to about 1250 mg/m^2 on days 1 and 8 or days 1 and 15 of a 21 day treatment cycle. The present invention contemplates administration of gemcitabine for one or more treatment cycles, for example, 1, 2, 3, 4, 5, 6, or more treatment cycles. Embodiments of the present invention include those wherein gemcitabine is administerd by IV infusion over 30 minutes. In certain embodiments about 1000

mg/m^2 is delivered by IV infusion over 30 minutes on days 1, 8, and 15 of each 28 day treatment cycle. It will be understood that any of the dose ranges indicated herein for treatment with gemcitabine can be combined with any of the dose ranges indicated herein for treatment with the antibody-drug conjugate compound provided that administration provides the desired therapeutic effect, *i.e.,* synergism.

[0103] In some particularly preferred examples of the present invention, administration of a synergistic amount of the therapeutic agents encompasses administering the antibody drug conjugate compound in a total range of about 0.5 mg/kg to about 5 mg/kg, about 0.6 mg/kg to about 5 mg/kg, about 0.6 mg/kg to about 2.7 mg/kg, about 0.8 mg/kg to about 2.7 mg/kg, about 1 mg/kg to about 5 mg/kg, about 1 mg/kg to about 4 mg/kg, about 1 mg/kg to about 3.5 mg/kg, about 1.5 mg/kg to about 3. 5 mg/kg. or even about 1.8 mg/kg to about 2.5 mg/kg over a 21 or 28 day treatment cycle, irrespective of the dosing schedule, in combination with administering gemcitabine at standard dosing schedules known in the art, e.g., about 800 mg/m^2 to about 1500 mg/m^2 at each gemcitabine administration during the treatment cycle, preferably about 1000 mg/m^2 to about 1250 mg/m^2 at each gemcitabine administration during the treatment cycle (e.g, 1-3 times during the 21 or 28 day treatment cycle).

[0104] In some particularly preferred examples of the present invention, administration of a synergistic amount of the therapeutic agents encompasses administering the antibody drug conjugate compound once during the treatment cycle (e.g., a 21 or 28 day treatment cycle) in a range of about 0.5 to about 2.7 mg/kg, about 0.6 mg/kg to about 2.7 mg/kg, about 0.6 mg/kg to about 2 mg/kg, about 0.6 mg/kg to about 1 mg/kg, about 0.8 mg/kg to about 2.7 mg/kg, about 0.8 mg/kg to about 2.0 mg/kg, about 1 mg/kg to about 2.7 mg/kg, about 1.5 mg/kg to about 2.7 mg/kg, or even more preferably about 1.0 mg/kg to about 2 mg/kg or about 1.5 mg/kg to about 2 mg/kg of the subject's body weight in combination with administering gemcitabine at standard dosing schedules known in the art, e.g., about 800 mg/m^2 to about 1500 mg/m^2 at each gemcitabine administration during the treatment cycle, preferably about 1000 mg/m^2 to about 1250 mg/m^2 at each gemcitabine administration during the treatment cycle (e.g, 1-3 times during the treatment cycle). For example, in one embodiment, administration of a synergistic amount of the therapeutic agents includes administering the antibody drug conjugate compound once during a 3 week treatment cycle (e.g., on day 1 of a 21 day treatment cycle) in a

range of about 0.5 mg/kg to about 2.7 mg/kg, about 0.6 mg/kg to about 2.7 mg/kg, about 0.8 mg/kg to about 2.0 mg/kg, about 1.5 mg/kg to about 2.7 mg/kg, or about 1.5 mg/kg to about 2 mg/kg of the subject's body weight in combination with administering gemcitabine on days 1 and 8 or days 1 and 15 of the 21 day treatment cycle in a range of about 800 mg/m^2 to about 1500 mg/m^2, preferably about 1000 mg/m^2 to about 1250 mg/m^2.

[0105] In some particularly preferred examples of the present invention, administration of a synergistic amount of the therapeutic agents encompasses administering the antibody drug conjugate compound three times during the treatment cycle (e.g., a 21 or 28 day treatment cycle) in a range of about 0.4 mg/kg to about 2 mg/kg, about 0.4 mg/kg to about 1.8 mg/kg, about 0.4 mg/kg to about 1 mg/kg, about 0.4 mg/kg to about 1.5 mg/kg at each administration in combination with administering gemcitabine at standard dosing schedules known in the art, e.g., about 800 mg/m^2 to about 1500 mg/m^2 at each gemcitabine administration during the treatment cycle, preferably about 1000 mg/m^2 to about 1250 mg/m^2 at each gemcitabine administration during the treatment cycle (e.g, 1-3 times during the treatment cycle). For example, in one embodiment, administration of a synergistic amount of the therapeutic agents includes administering the antibody drug conjugate compound on days 1, 8 and 15 of a 28 day cycle in a range of about 0.4 mg/kg to about 2 mg/kg, about 0.4 mg/kg to about 1.8 mg/kg, about 0.4 mg/kg to about 1 mg/kg of the subject's body weight, about 0.4 mg/kg to about 1.5 mg/kg of the subject's body weight at each administration in combination with administering gemcitabine on days 1, 8 and 15 of a 28 day cycle in a range of about 800 mg/m^2 to about 1250 mg/m^2, preferably about 1000 mg/m^2 to about 1250 mg/m^2 at each administration.

[0106] In some particularly preferred examples of the present invention, administration of a synergistic amount of the therapeutic agents encompasses administering the antibody drug conjugate compound two times during the treatment cycle (e.g., a 21 or 28 day treatment cycle) in a range of about 0.4 mg/kg to about 2.0 mg/kg, about 0.4 mg/kg to about 1.8 mg/kg, about 0.4 mg/kg to about 1 mg/kg, about 0.4 mg/kg to about 1.5 mg/kg at each administration in combination with administering gemcitabine at standard dosing schedules known in the art, e.g., about 800 mg/m^2 to about 1500 mg/m^2 at each gemcitabine administration during the treatment cycle, preferably about 1000 mg/m^2 to about 1250 mg/m^2 at each

gemcitabine administration during the treatment cycle (e.g, 1-3 times during the treatment cycle). For example, administration of a synergistic amount of the therapeutic agents includes administering the antibody drug conjugate compound on days 1 and 15 of a 28 day cycle in a range of about 0.4 mg/kg to about 2 mg/kg, about 0.4 mg/kg to about 1.8 mg/kg, about 0.4 mg/kg to about 1 mg/kg of the subject's body weight, about 0.4 mg/kg to about 1.5 mg/kg of the subject's body weight at each administration in combination with administering gemcitabine on days 1, 8 and 15 of a 28 day cycle in a range of about 800 mg/m^2 to about 1250 mg/m^2, preferably about 1000 mg/m^2 to about 1250 mg/m^2 at each administration.

[0107] In embodiments of the present invention wherein treatment comprises administration of the antibody-drug conjugate compound and the chemotherapeutic regimen comprising bleomycin, vinblastine and dacarbazine or doxorubicin, bleomycin, vinblastine and dacarbazine (ABVD), administration of the antibody-drug conjugate compound can be on the same or different days as administration of the chemotherapeutic regimen provided that administration provides the desired therapeutic effect. The present invention encompasses, for example, embodiments wherein the chemotherapeutic regimen is administered on days 1 and 15 of a four week cycle. In certain embodiments, both the chemotherapeutic regimen and the antibody-drug conjugate compound are administered on days 1 and 15 of a four week cycle. In other embodiments, the chemotherapeutic regimen will be administered on days 1 and 15 of a four week cycle and the antibody-drug conjugate compound will be administered on days 1, 8 and 15 of a four week cycle or on day 1 of a three or four week cycle. Other administration schedules are encompassed by the present methods. Methods of administering the drugs bleomycin, vinblastine and dacarbazine or doxorubicin, bleomycin, vinblastine and dacarbazine in a chemotherapeutic regimen for the treatment of Hodgkin lymphoma are known. Typically, administration is on days 1 and 15 of a 28 day cycle and doxorubicin is administered at a dosage of 25 mg/m^2, bleomycin is administered at a dosage of 10 U/ m^2, vinblastine is administered at a dosage of 6 mg/ m^2, and dacarbazine is administered at a dosage of 375 mg/ m^2. Embodiments of the present invention include those wherein the drugs are administered at the levels currently indicated in the art for the treatment of HL. Embodiments of the present invention include those wherein the drugs are administered at lower or higher levels than currently indicated in the art for the treatment of HL provided that administration provides the desired therepauetic effect.

In certain instances, dosage levels can be reduced when combined with additional therapeutic agents. Embodiments of the present invention include, for example, those wherein the ABVD regimen is administered at about the MTD, maximum tolerated dose. In certain embodiments, doxorubicin is administered in a range of 0-35 mg/m^2, 10-30 mg/m^2 or 10-25 mg/m^2 at each administration, *e.g.,* on days 1 and 15 of a 28 day treatment cycle; bleomycin is administered in a range of 2 to 15 U/m^2, 5 to 15 U/m^2, or 5 to 10 U/m^2 at each administration, *e.g.,* on days 1 and 15 of a 28 day treatment cycle, vinblasine is administered in range of 1-8 mg/m^2, 2-6 mg/m^2 or 3-6 mg/m^2 at each administration, *e.g.,* on days 1 and 15 of a 28 day treatment cycle, and dacarbazine is administered in a range of 100-450 mg/m^2, 150-375 mg/m^2, 200-375 mg/m^2 or 300-375 mg/m^2 at each administration, *e.g.,* on days 1 and 15 of a 28 day treatment cycle provided that administration provides the desired therapeutic effect. The present invention contemplates administration of the ABVD regimen for one or more treatment cycles, for example, 1, 2, 3, 4, 5, 6, or more treatment cycles. It will be understood that any of the dose ranges indicated herein for treatment with doxorubicin, bleomycin, vinblastine and dacarbazine can be combined with any of the dose ranges indicated herein for treatment with the antibody-drug conjugate compound provided that administration provides the desired therapeutic effect.

[0108] In some particularly preferred examples of the present invention, administration of a synergistic amount of the therapeutic agents encompasses administering the antibody drug conjugate compound in a total range of about 0.5 mg/kg to about 5 mg/kg, about 0.6 mg/kg to about 5 mg/kg, about 0.6 mg/kg to about 2.7 mg/kg, about 0.8 mg/kg to about 2.7 mg/kg, about 1 mg/kg to about 5 mg/kg, about 1 mg/kg to about 4 mg/kg, about 1 mg/kg to about 3.5 mg/kg, about 1.5 mg/kg to about 3. 5 mg/kg. or even about 1.8 mg/kg to about 2.5 mg/kg over a 21 or 28 day treatment cycle, irrespective of the dosing schedule, in combination with administering ABVD at standard dosing schedules known in the art.

[0109] In some particularly preferred examples of the present invention, administration of a synergistic amount of the therapeutic agents encompasses administering the antibody drug conjugate compound once during the treatment cycle (e.g., a 21 or 28 day treatment cycle) in a range of about 0.5 to about 2.7 mg/kg, about 0.6 mg/kg to about 2.7 mg/kg, about 0.6 mg/kg to about 2 mg/kg, about 0.6 mg/kg to about 1 mg/kg, about 0.8 mg/kg to about 2.7 mg/kg, about 0.8 mg/kg to about 2.0 mg/kg, about 1 mg/kg to about 2.7 mg/kg, about 1.5 mg/kg to about 2.7

mg/kg, or even more preferably about 1.0 mg/kg to about 2 mg/kg or about 1.5 mg/kg to about 2 mg/kg of the subject's body weight, in combination with administering ABVD at standard dosing schedules known in the art.

[0110] In some particularly preferred examples of the present invention, administration of a synergistic amount of the therapeutic agents encompasses administering the antibody drug conjugate compound three times during the treatment cycle (e.g., a 21 or 28 day treatment cycle) in a range of about 0.4 mg/kg to about 2 mg/kg, about 0.4 mg/kg to about 1.8 mg/kg, about 0.4 mg/kg to about 1 mg/kg, about 0.4 mg/kg to about 1.5 mg/kg at each administration in combination with administering ABVD at standard dosing schedules known in the art.

[0111] In some particularly preferred examples of the present invention, administration of a synergistic amount of the therapeutic agents encompasses administering the antibody drug conjugate compound two times during the treatment cycle (e.g., a 21 or 28 day treatment cycle) in a range of about 0.4 mg/kg to about 2.0 mg/kg, about 0.4 mg/kg to about 1.8 mg/kg, about 0.4 mg/kg to about 1 mg/kg, about 0.4 mg/kg to about 1.5 mg/kg at each administration, in combination with administering ABVD at standard dosing schedules known in the art.

I. Pharmaceutical Compositions

[0112] Various delivery systems are known and can be used to administer the antibody-drug conjugate compounds and the chemotherapeutic agents. Methods of introduction include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, and subcutaneous routes. Administration can be, for example by infusion or bolus injection. In certain preferred embodiments, administration of both the chemotherapeutic agent and the antibody-drug conjugate compound is by infusion.

[0113] The antibody-drug conjugate compound and chemotherapeutic agents can be administered as pharmaceutical compositions comprising one or more pharmaceutically compatible ingredients. For example, the pharmaceutical composition typically includes one or more pharmaceutical carriers (*e.g.*, sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like). Water is a more typical carrier when the pharmaceutical composition is administered

intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients are known in the art. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. The formulations correspond to the mode of administration.

[0114] In typical embodiments, the pharmaceutical composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the pharmaceutical can also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the pharmaceutical is to be administered by infusion, it can be dispensed, for example, with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the pharmaceutical is administered by injection, an ampoule of sterile water for injection or saline can be, for example, provided so that the ingredients can be mixed prior to administration.

J. Subjects

[0115] The methods of the present invention encompass administering combination therapy to a subject for the treatment of Hodgkin lymphoma.

[0116] The subjects to be treated with the methods of the present invention are those that have been diagnosed with Hodgkin lymphoma or are suspected of having Hodgkin lymphoma. Diagnosis can be by methods known in the art, including, for example, lymph node biopsy. After Hodgkin lymphoma is diagnosed, if desired, a subject can be classified according to stage of disease using one of the known classification schemes. The Cotswolds staging classification scheme is one such classification scheme. Briefly, stage I can be characterized by involvement of a single lymph node region or lymphoid structure; stage II can be characterized by

involvement of two or more lymph node regions or lymph node structures on the same side of the diaphragm; stage III can be characterized by involvement of lymph node regions or lymph node structures on both sides of the diaphragm; and stage IV can be characterized by diffuse or disseminated involvement of one or more extranodal organs or tissue beyond that designated E, with or without lymph node involvement. The designation E refers to extranodal contiguous extension that can be encompassed within an irradiation field appropriate for nodal disease of the same anatomic extent. Subjects in stages I or II can have a favorable or unfavorable prognosis depending on the presence or absence of certain clinical features. For the purposes of the present invention, subjects with early stage disease are classified in Stage I or II whereas subjects with advanced stage disease are classified in Stages III or IV. The methods of the present invention can be used to treat a subject classified in any one of the four stages of disease, including a subject with advanced stage disease.

[0117] The methods of the present invention encompass treating a subject who is newly diagnosed and has not previously been treated for HL.

[0118] The methods of the present invention also can be used to treat subjects with refractory and/or relapsed Hodgkin lymphoma.

[0119] A subject with refractory Hodgkin lymphoma is a subject who does not respond to therapy for HL, *i.e.,* the subject continues to experience disease progresssion despite therapy.

[0120] A subject with relapsed Hodgkin lymphoma is a subject who has responded to therapy for HL at one point, but has had a reoccurence or further progression of disease following the response.

[0121] The methods of the present invention also encompass treating a subject who has previously been treated with a first-line chemotherapy regimen for Hodgkin lymphoma or a subject who has been treated with both a first-line chemotherapy regimen and/or a salvage chemotherapy regimen. First line chemotherapeutic regimens for Hodgkin lymphoma include, for example, the ABVD regimen (Bonadonna and Santoro, Cancer Treat Rev 1982;9:21-35), the BEACOPP regimen (Diehl et al., N Engl J Med 2003;348:2386-2395), the escalated BEACOPP regimen (Diehl et al., N Engl J Med 2003;348:2386-2395), the MOPP regimen (Devita et al., Ann Inter Med 1970:73:881-895), and the Stanford V regimen (Horning et al., J Clin Oncol 2000;18:972-980). Salvage chemotherapy regimens include, for example, the ESHAP regimen (Aparicio et al., Ann Ocol 1999;10:593-595), the modified Stanford

V regimen (Aviles et al., Med Oncol 2001;18:261-267), the GDP regimen (Baetz et al., Ann Oncol 2003;14:1762-1767), the Mini-Beam regimen (Colwill et al., J Clin Oncol 1995;13:396-402, Fernandez-Jimenez et al., Haematologica 1999;84:1007-1011), the MIME regimen (Enblad et al., Eur J Haematol 1998;60:166-171), the MINE regimen (Ferme et al., Ann Oncol 1995;6:543-549), the IEE regimen (Jackson et al., Leuk Lymphoma 2000;37:561-570), the DHAP regimen (Josting et al., Ann Oncol 2002;13:1628-1635), the ICE regimen (Moskowitz et al., Semin Oncol 2004;31(suppl):54-59), the IIVP regimen (Oyan et al., Biol Blood Marrow Transplant 2005;11:688-697), the IVE regimen (Proctor et al., Eur J Haematol 2001;64(suppl):28-32), the VIP regimen (Ribrag et al., Bome Marrow Transplant 1998;21:969-974), the ASHAP regimen (Rodriguez et al., Blood 1999;93:3632-3636), the Dexa-BEAM regimen (Schmitz et al., Lancet 2002;359:2065-2071), the CEP regimen (Szanto et al., Oncology 1991;48:456-458), the CN30P regimen (Walewski et al., Med Oncol. 2000;17:195-202), and the MVC regimen (Wiernik et al., Cancer J Sci Am 1998;4:254-260).

[0122] The methods of the present invention also encompass treating a subject who has previously undergone a stem cell transplant.

K. Anti-CD30 Antibodies

[0123] Anti-CD30 antibodies suitable for use in accordance with the present compositions and methods include any antibody that specifically binds to the CD30 antigen. Anti-CD30 antibodies are preferably monoclonal and can include, for example, chimeric (*e.g.*, having a human constant region and mouse variable region), humanized, or human antibodies; single chain antibodies; or the like. The immunoglobulin molecules can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

[0124] In certain embodiments, the antibody is an antigen-binding antibody fragment such as, for example, a Fab, a F(ab'), a $F(ab')_2$, a Fd chain, a single-chain Fv (scFv), a single-chain antibody, a disulfide-linked Fv (sdFv), a fragment comprising either a V_L or V_H domain, or fragments produced by a Fab expression library, or a CD30 -binding fragment of any of the above antibodies. Antigen-binding antibody

fragments, including single-chain antibodies, can comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, CH3 and CL domains. Also, antigen-binding fragments can comprise any combination of variable region(s) with a hinge region, CH1, CH2, CH3 and CL domains. Typically, the antibodies are human, rodent (*e.g.*, mouse and rat), donkey, sheep, rabbit, goat, guinea pig, camelid, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries, from human B cells, or from animals transgenic for one or more human immunoglobulin (see, for example in U.S. Patent Nos. 5,939,598 and 6,111,166).

[0125] The antibodies may be monospecific, bispecific, trispecific, or of greater multispecificity (*See*, *e.g.*, PCT publications WO 93/17715; WO 92/08802; WO 91/00360; and WO 92/05793; Tutt *et al.*, 1991, *J Immunol* 147:60-69; U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; and 5,601,819; Kostelny *et al.*, 1992, *J Immunol* 148:1547-1553.)

[0126] Exemplary anti-CD30 antibodies include, but are not limited to, humanized or chimeric AC10 or HeFi-1 antibodies. Accordingly, an exemplary anti-CD30 antibody comprises one or more CDRs of murine HeFi-1 (SEQ ID NO:20, SEQ ID NO:22; SEQ ID NO:24; SEQ ID NO:28, SEQ ID NO:30 or SEQ ID NO:32) or murine AC10 (SEQ ID NO:4; SEQ ID NO:6; SEQ ID NO:8; SEQ ID NO:12; SEQ ID NO:14; or SEQ ID NO:16). In some embodiments, the anti-CD30 antibody comprises one/or one or more variable regions of murine HeFi-1 (SEQ ID NO:18 or SEQ ID NO:26) or murine AC10 (SEQ ID NO:2 or SEQ ID NO:10). A table indicating the region of AC10 or HeFi-1 to which each SEQ ID NO corresponds to is provided below:

Table 1

MOLECULE	NUCLEOTIDE OR AMINO ACID	SEQ ID NO
AC10 Heavy Chain Variable Region	Nucleotide	1
AC10 Heavy Chain Variable Region	Amino Acid	2
AC10 Heavy Chain-CDR1(H1)	Nucleotide	3
AC 10 Heavy Chain-CDR1(H1)	Amino Acid	4
AC 10 Heavy Chain-CDR2(H2)	Nucleotide	5
AC 10 Heavy Chain-CDR2(H2)	Amino Acid	6
AC 10 Heavy Chain-CDR3(H3)	Nucleotide	7

MOLECULE	NUCLEOTIDE OR AMINO ACID	SEQ ID NO
AC 10 Heavy Chain-CDR3(H3)	Amino Acid	8
AC 10 Light Chain Variable Region	Nucleotide	9
AC 10 Light Chain Variable Region	Amino Acid	10
AC 10 Light Chain-CDR1(L1)	Nucleotide	11
AC 10 Light Chain-CDR1(L1)	Amino Acid	12
AC 10 Light Chain-CDR2(L2)	Nucleotide	13
AC 10 Light Chain-CDR2(L2)	Amino Acid	14
AC 10 Light Chain-CDR3(L3)	Nucleotide	15
AC 10 Light Chain-CDR3(L3)	Amino Acid	16
HeFi-1 Heavy Chain Variable Region	Nucleotide	17
HeFi-1 Heavy Chain Variable Region	Amino Acid	18
HeFi-1 Heavy Chain-CDR1(H1)	Nucleotide	19
HeFi-1 Heavy Chain-CDR1(H1)	Amino Acid	20
HeFi-1 Heavy Chain-CDR2(H2)	Nucleotide	21
HeFi-1 Heavy Chain-CDR2(H2)	Amino Acid	22
HeFi-1 Heavy Chain-CDR3(H3)	Nucleotide	23
HeFi-1 Heavy Chain-CDR3(H3)	Amino Acid	24
HeFi-1 Light Chain Variable Region	Nucleotide	25
HeFi-1 Light Chain Variable Region	Amino Acid	26
HeFi-1 Light Chain-CDR1(L1)	Nucleotide	27
HeFi-1 Light Chain-CDR1(L1)	Amino Acid	28
HeFi-1 Light Chain-CDR2(L2)	Nucleotide	29
HeFi-1 Light Chain-CDR2(L2)	Amino Acid	30
HeFi-1 Light Chain-CDR3(L3)	Nucleotide	31
HeFi-1 Light Chain-CDR3(L3)	Amino Acid	32
Human gamma I constant region	Amino Acid	33
Human kappa constant region	Amino Acid	34

[0127] Exemplary anti-CD30 antibodies include functional derivatives or analogs of AC10 and HeFi-1. As used herein, the term "functional" in this context indicates that the functional derivate or analog of AC10 and HeFi-1 is capable of binding to CD30.

[0128] In some embodiments, anti-CD30 antibodies not only immunospecifically binds CD30 but also can exert cytostatic and/or cytotoxic effect on malignant cells in HL wherein the cytostatic or cytotoxic effect is complement-independent and can be achieved in the absence of (i) conjugation to a cytostatic or cytotoxic agent and (ii) effector cells.

[0129] The anti-CD30 antibodies may be described or specified in terms of the particular CDRs they comprise. In some embodiments, the antibodies comprise the CDRs of AC10 and/or HeFi-1. In some embodiments, the antibodies are chimeric or humanized forms of AC10 or HeFi-1. The invention encompasses an

antibody comprising a heavy or light chain variable domain, said variable domain comprising (a) a set of three CDRs, in which said set of CDRs are from murine monoclonal antibody AC10 or HeFi-1, and (b) a set of four framework regions, in which said set of framework regions differs from the set of framework regions in murine monoclonal antibody AC10 or HeFi-1, respectively, and in which said antibody immunospecifically binds CD30.

[0130] In a specific embodiment, the invention encompasses an antibody comprising a heavy chain variable domain, said variable domain comprising (a) a set of three CDRs, in which said set of CDRs comprises SEQ ID NO:4, 6, or 8 or comprises amino acid sequences that are substantially identical to the amino acid sequences set forth in SEQ ID NO:4, 6, or 8 and (b) a set of four framework regions, in which said set of framework regions differs from the set of framework regions in murine monoclonal antibody AC10, and in which said antibody immunospecifically binds CD30.

[0131] In a specific embodiment, the invention encompasses an antibody comprising a heavy chain variable domain, said variable domain comprising (a) a set of three CDRs, in which said set of CDRs comprises SEQ ID NO:20, 22 or 24 or comprises amino acid sequences that are substantially identical to the amino acid sequences set forth in SEQ ID NO:20, 22, or 24 and (b) a set of four framework regions, in which said set of framework regions differs from the set of framework regions in murine monoclonal antibody HeFi-1, and in which said antibody immunospecifically binds CD30.

[0132] In a specific embodiment, the invention encompasses an antibody comprising a light chain variable domain, said variable domain comprising (a) a set of three CDRs, in which said set of CDRs comprises SEQ ID NO:12, 14 or 16 or comprises amino acid sequences that are substantially identical to the amino acid sequences set forth in SEQ ID NO:12, 14, or 16, and (b) a set of four framework regions, in which said set of framework regions differs from the set of framework regions in murine monoclonal antibody AC10, and in which said antibody immunospecifically binds CD30.

[0133] In a specific embodiment, the invention encompasses an antibody comprising a light chain variable domain, said variable domain comprising (a) a set of three CDRs, in which said set of CDRs comprises SEQ ID NO:28, 30, or 32 or

comprises amino acid sequences that are substantially identical to the amino acid sequences set forth in SEQ ID NO:28, 30, or 32, and (b) a set of four framework regions, in which said set of framework regions differs from the set of framework regions in murine monoclonal antibody HeFi-1, and in which said antibody immunospecifically binds CD30.

[0134] The present invention encompasses embodiments wherein a chimeric AC10 antibody comprises the heavy chain variable region set forth in SEQ ID NO:2, the light chain variable region set forth in SEQ ID NO:10, the human gamma I constant region set forth in SEQ ID NO:33 or amino acids 1 to 329 of SEQ ID NO:33 and the human kappa constant region set forth in SEQ ID NO:34.

[0135] Additionally, the antibodies can also be described or specified in terms of their primary structures. Anti-CD30 antibodies having at least 80%, at least 85%, at least 90%, at least 95% and most preferably at least 98% identity (as calculated using methods known in the art and described herein) to the variable regions of murine AC10 or HeFi-1 are also included in the present invention. Antibodies of the present invention may also be described or specified in terms of their binding affinity to CD30. Preferred binding affinities include those with a dissociation constant or Kd less than 5 X 10^{-6} M, 10^{-6} M, 5 X 10^{-7} M, 10^{-7} M, 5 X 10^{-8} M, 10^{-8} M, 5 X 10^{-9} M, 10^{-9} M, 5 X 10^{-10} M, 10^{-10} M, 5 X 10^{-11} M, 10^{-11} M, 5 X 10^{-12} M, 10^{-12} M, 5 X $^{-13}$ M, 10^{-13} M, 5 X 10^{-14} M, 10^{-14} M, 5 X 10^{-15} M, or 10^{-15} M.

[0136] The antibodies can be purified, for example by affinity chromatography with the CD30 antigen. In certain embodiments, the antibody is at least 50%, at least 60%, at least 70% or at least 80% pure. In other embodiments, the antibody is more than 85% pure, more than 90% pure, more than 95% pure or more than 99% pure.

[0137] The antibodies also include antibodies that are modified, *e.g.,* by the attachment of any type of molecule to the antibody such that attachment does not prevent the antibody from binding to CD30. For example, but not by way of limitation, the term "antibody" includes antibodies that have been modified, *e.g.*, by glycosylation, deglycosylation, acetylation, pegylation, phosphylation, amidation, derivatization by known protecting/blocking groups, linkage to a cellular ligand or other protein, *etc*. Any of numerous chemical modifications may be carried out by

known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, *etc.*

[0138] The antibodies of the present invention can be generated by any suitable method known in the art. Polyclonal antibodies to CD30 can be produced by various procedures well known in the art. For example, CD30 can be administered to various host animals including, but not limited to, rabbits, mice, rats, and the like, to induce the production of sera containing polyclonal antibodies specific for the protein. Various adjuvants may be used to increase the immunological response, depending on the host species.

[0139] Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow *et al.*, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed., 1988); Hammerling, *et al.*, in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties).

[0140] Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art. In a non-limiting example, mice can be immunized with CD30 or a cell expressing CD30 or a fragment or derivative thereof. Once an immune response is detected, *e.g.*, antibodies specific for CD30 are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the American Type Culture Collection, Rockville, MD (ATCC). Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding CD30. Ascites fluid, which generally contains high levels of antibodies, can be generated by injecting mice with positive hybridoma clones.

[0141] Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen

of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind to CD30.

[0142] Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and $F(ab')_2$ fragments may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce $F(ab')_2$ fragments). $F(ab')_2$ fragments contain the variable region, the light chain constant region and the CH 1 domain of the heavy chain.

[0143] Antibodies can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the nucleic acid sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (*e.g.*, human or murine). In particular, DNA sequences encoding V_H and V_L domains are amplified from animal cDNA libraries (*e.g.*, human or murine cDNA libraries of lymphoid tissues). The DNA encoding the V_H and V_L domains are recombined together with an scFv linker by PCR and cloned into a phagemid vector (*e.g.*, p CANTAB 6 or pComb 3 HSS). The vector is electroporated in *E. coli* and the *E. coli* is infected with helper phage. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody (dsFv) domains recombinantly fused to either the phage gene III or gene VIII protein. Phage expressing an antigen binding domain that binds to CD30 or an AC10 or HeFi-1 variable region can be selected or identified with antigen *e.g.*, using labeled antigen or antigen bound or captured to a solid surface or bead. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman *et al.*, 1995, J. Immunol. Methods 182:41-50; Ames *et al.*, 1995, J. Immunol. Methods 184:177-186; Kettleborough *et al.*, 1994, Eur. J. Immunol. 24:952-958; Persic *et al.*, 1997, Gene 187:9-18; Burton *et al.*, 1994, Advances in Immunology, 191-280; PCT Application No. PCT/GB91/O1 134; PCT Publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/1 1236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225;

5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

[0144] As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, *e.g.*, as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and $F(ab')_2$ fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax *et al.*, BioTechniques 1992, 12(6):864-869; and Sawai *et al.*, 1995, AJRI 34:26-34; and Better *et al.*, 1988, Science 240:1041-1043 (said references incorporated by reference in their entireties).

[0145] Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston *et al.*, 1991, Methods in Enzymology 203:46-88 ;Shu *et al.*, 1993, PNAS 90:7995-7999; and Skerra *et al.*, 1988, Science 240:1038-1040. For some uses, including *in vivo* use of antibodies in humans and *in vitro* proliferation or cytotoxicity assays, it is preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. *See e.g.*, Morrison, Science, 1985, 229:1202 ; Oi *et al.*, 1986, BioTechniques 4:214; Gillies *et al.*, 1989, J. Immunol. Methods 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more CDRs from the non-human species and framework and constant regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, *e.g.*, by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence

comparison to identify unusual framework residues at particular positions. (*See*, *e.g.*, Queen *et al.*, U.S. Patent No. 5,585,089; Riechmann *et al.*, , 1988, Nature 332:323 , which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 9 1/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology, 1991, 28(4/5):489-498; Studnicka *et al.*, 1994, Protein Engineering 7(6):805-814; Roguska. *et al.*, 1994, PNAS 91:969-973), and chain shuffling (U.S. Patent No. 5,565,332).

[0146] Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

[0147] Human antibodies can also be produced using transgenic mice which express human immunoglobulin genes. For an overview of this technology for producing human antibodies, *see*, Lonberg and Huszar, 1995, Int. Rev. Immunol. 13:65-93. For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, *see*, *e.g.*, PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Amgen. (Thousand Oaks, CA) and Medarex (Princeton, NJ) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

[0148] Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, *e.g.*, a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers *et al.*, 1994, Bio/technology 12:899-903).

[0149] Antibodies for use in the present invention include chimeric and humanized AC10 as well as chimeric and humanized HeFi-1. Antibodies for use in the present invention include those that competitively inhibit binding of murine AC10 or HeFi-1 to CD30 as determined by any method known in the art for determining competitive binding. For example, the antibody can inhibit binding of AC10 or HeFi-1 to CD30 by at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, or even at least 95%. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled CD30 (*e.g.*, ^{3}H or ^{125}I) with the antibody of interest in the presence of increasing amounts of unlabeled CD30, and the detection of the antibody bound to the labeled CD30. The affinity of the antibody for CD30 and the binding off-rates can then be determined from the data by Scatchard plot analysis. Competition with a second antibody (such as AC10 or HeFi-1) can also be determined using radioimmunoassays. In this case, CD30 is incubated with the antibody of interest conjugated to a labeled compound (*e.g.*, ^{3}H or ^{125}I) in the presence of increasing amounts of an unlabeled second antibody. Antibodies for use in the present invention also include antibodies other than chimeric or humanized AC10 or HeFi-1 that specifically bind to CD30.

[0150] One method which detects protein interactions *in vivo*, the two-hybrid system, is described in detail for illustration purposes only and not by way of limitation. One version of this system has been described (Chien *et al.*, 1991, Proc. Natl. Acad. Sci. USA, 88:9578-9582) and is commercially available from Clontech (Palo Alto, CA).

[0151] Once a CD30-binding protein is identified, if desired, its ability (alone or when multimerized or fused to a dimerization or multimerization domain) to elicit a cytostatic or cytotoxic effect on HL cells can be determined by contacting a culture of an HL cell line, such as L428, L450, HLLM2 or KM-H2, with the protein. Culture conditions are most preferably about 5,000 cells in a culture area of about 0.33 cm^2, and the contacting period being approximately 72 hours. The culture is then exposed to 0.5 μCi of ^{3}H-thymidine during the final 8 hours of the 72-hour period and the incorporation of ^{3}H-thymidine into cells of the culture is measured. The protein has a cytostatic or cytotoxic effect on the HL cell line if the cells of the culture have reduced ^{3}H-thymidine incorporation compared to cells of the same cell line cultured under the same conditions but not contacted with the protein. There are many other

cytotoxicity assays known to those of skill in the art. Any one of them can be used in the present methods.

[0152] The anti-CD30 antibodies that are useful in the present methods can be produced by any method known in the art for the synthesis of proteins, typically, *e.g.*, by recombinant expression techniques. Recombinant expression of an antibody or derivative thereof that binds to CD30 and depletes or inhibits the proliferation of CD30-expressing cells can include construction of an expression vector containing a nucleic acid that encodes the antibody or derivative thereof. Once a nucleic acid encoding such a protein has been obtained, the vector for the production of the protein molecule may be produced by recombinant DNA technology using techniques well known in the art. Standard techniques such as, for example, those described in Sambrook and Russell, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 3rd ed., 2001); Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2nd ed., 1989); *Short Protocols in Molecular Biology* (Ausubel *et al.*, John Wiley & Sons, New York, 4th ed., 1999); and Glick & Pasternak, *Molecular Biotechnology: Principles and Applications of Recombinant DNA* (ASM Press, Washington, D.C., 2nd ed., 1998) can be used for recombinant nucleic acid methods, nucleic acid synthesis, cell culture, transgene incorporation, and recombinant protein expression.

[0153] For example, for recombinant expression of an anti-CD30 antibody, an expression vector may encode a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. An expression vector may include, for example, the nucleotide sequence encoding the constant region of the antibody molecule (*see, e.g.*, PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464), and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain. The expression vector is transferred to a host cell by conventional techniques, and the transfected cells are then cultured by conventional techniques to produce the anti-CD30 antibody. In typical embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains can be co-expressed in the host cell for expression of the entire immunoglobulin molecule.

[0154] A variety of prokaryotic and eukaryotic host-expression vector systems can be utilized to express an anti-CD30 antibody or derivative thereof. Typically, eukaryotic cells, particularly for whole recombinant anti-CD30 antibody molecules, are used for the expression of the recombinant protein. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus, is an effective expression system for the production of anti-CD30 antibodies (*see, e.g.*, Foecking *et al.*, 1986, *Gene* 45:101; Cockett *et al.*, 1990, *Bio/Technology* 8:2). Anti-CD30 antibodies can also be expressed using the CHEF system. (*See*, *e.g.*, U.S. Patent No. 5,888,809.)

[0155] Other host-expression systems include, for example, plasmid-based expression systems in bacterial cells (*see, e.g.*, Ruther *et al.*, 1983, *EMBO* 1,2:1791; Inouye & Inouye, 1985, *Nucleic Acids Res.* 13:3101-3109; Van Heeke & Schuster, 1989, *J. Biol. Chem.* 24:5503-5509); insect systems such as, *e.g.*, the use of *Autographa californica* nuclear polyhedrosis virus (AcNPV) expression vector in *Spodoptera frugiperda* cells; and viral-based expression systems in mammalian cells, such as, *e.g.*, adenoviral-based systems (*see*, *e.g.*, Logan & Shenk, 1984, *Proc. Natl. Acad. Sci. USA* 81:355-359; Bittner *et al.*, 1987, *Methods in Enzymol.* 153:51-544).

[0156] In addition, a host cell strain can be chosen that modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing (*e.g.*, glycosylation, phosphorylation, and cleavage) of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript and gene product can be used. Such mammalian host cells include, for example, CHO (*e.g.*, DG44 and CHO-S), VERO, BHK, HeLa, COS, MDCK, 293, 3T3, and W138.

[0157] A stable expression system is typically used for long-term, high-yield production of recombinant anti-CD30 antibody. For example, cell lines that stably express the anti-CD30 antibody or derivative thereof can be engineered by transformation of host cells with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites) and a selectable marker, followed by growth of the transformed

cells in a selective media. The selectable marker confers resistance to the selection and allows cells to stably integrate the DNA into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. A number of selection systems can be used, including, for example, the herpes simplex virus thymidine kinase, hypoxanthineguanine phosphoribosyltransferase, and adenine phosphoribosyltransferase genes, which can be employed in tk^-, $hgprt^-$ or $aprt^-$ cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate; gpt, which confers resistance to mycophenolic acid; neo, which confers resistance to the aminoglycoside G-418; and hygro, which confers resistance to hygromycin. Methods commonly known in the art of recombinant DNA technology can be routinely applied to select the desired recombinant clone, and such methods are described, for example, in *Current Protocols in Molecular Biology* (Ausubel *et al.* eds., John Wiley & Sons, N.Y., 1993); Kriegler, *Gene Transfer and Expression, A Laboratory Manual* (Stockton Press, N.Y., 1990); *Current Protocols in Human Genetics* (Dracopoli *et al.* eds., John Wiley & Sons, N.Y., 1994, Chapters 12 and 13); and Colberre-Garapin *et al.*, 1981, *J. Mol. Biol.* 150:1.

[0158] Once an anti-CD30 antibody has has been produced (*e.g.*, by an animal, chemical synthesis, or recombinant expression), it can be purified by any suitable method for purification of proteins, including, for example, by chromatography (*e.g.*, ion exchange or affinity chromatography (such as, for example, Protein A chromatography for purification of antibodies having an intact Fc region), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. An anti-CD30 antibody can, for example, be fused to a marker sequence, such as a peptide, to facilitate purification by affinity chromatography. Suitable marker amino acid sequences include, *e.g.*, a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), and the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson *et al.*, 1984, *Cell* 37:767), and the "flag" tag.

L. Antibody-Drug Conjugate Units

[0159] The methods described herein encompass the use of antibodies that (a) specifically bind to CD30 and (b) are conjugated to an auristatin compound. The antibody-drug conjugate compounds comprise an anti-CD30 antibody, covalently linked to at least one drug unit wherein the drug unit is an auristatin compound. The Drug units can be covalently linked directly or via a Linker unit (-LU-).

[0160] In some embodiments, the antibody-drug conjugate compound has the following formula:

$$L - (LU-D)_p \qquad \textbf{(I)}$$

or a pharmaceutically acceptable salt or solvate thereof; wherein:

L is the antibody unit, *i.e.,* anti-CD30 antibody (including anti-CD30 antibody fragments), and

(LU-D) is a Linker unit-Drug unit moiety, wherein:

LU- is a Linker unit, and

-D is the auristatin compound having cytostatic or cytotoxic activity against a target cell; and

p is an integer from 1 to about 20.

[0161] In some embodiments, p ranges from 1 to about 10, 1 to about 9, 1 to about 8, 1 to about 7, 1 to about 6, 1 to about 5, 1 to about 4, 1 to about 3, or 1 to about 2. In some embodiments, p ranges from 2 to about 10, 2 to about 9, 2 to about 8, 2 to about 7, 2 to about 6, 2 to about 5, 2 to about 4 or 2 to 3. In other embodiments, p is 1, 2, 3, 4, 5 or 6. In some embodiments, p is 2 or 4.

[0162] In some embodiments, the antibody-drug conjugate compound has the following formula:

$$L - (A_a-W_w-Y_y-D)_p \qquad \textbf{(II)}$$

or a pharmaceutically acceptable salt or solvate thereof;

wherein:

L is the antibody unit, i.e., anti-CD30 antibody (including anti-CD30 antibody fragments); and

$-A_a-W_w-Y_y-$ is a Linker unit (LU), wherein:

-A- is a Stretcher unit,

a is 0 or 1,

each -W- is independently an Amino Acid unit,

w is an integer ranging from 0 to 12,

-Y- is a self-immolative spacer unit,

y is 0, 1 or 2;

-D is an auristatin compound having cytostatic or cytotoxic activity against the target cell; and

p is an integer from 1 to about 20.

[0163] In some embodiments, a is 0 or 1, w is 0 or 1, and y is 0, 1 or 2. In some embodiments, if w is 1 to 12, then y is 1 or 2. In some embodiments, w is 2 to 12 and y is 1 or 2. In some embodiments, w is 0, y is 0, and a is 1. In some embodiments, a is 0 or 1, w is 0 or 1, and y is 0 or 1. In some embodiments, p ranges from 1 to about 10, 1 to about 9, 1 to about 8, 1 to about 7, 1 to about 6, 1 to about 5, 1 to about 4, 1 to about 3, or 1 to 2. In some embodiments, p ranges from 2 to about 8, 2 to about 7, 2 to about 6, 2 to about 5, 2 to about 4 or 2 to 3. In other embodiments, p is 1, 2, 3, 4, 5 or 6. In some embodiments, p is 2 or 4.

[0164] The drug loading is represented by p, the average number of drug molecules per antibody in a molecule. Drug loading may range from 1 to 20 drugs (D) per antibody. The average number of drugs per antibody in preparation of conjugation reactions may be characterized by conventional means such as mass spectroscopy, ELISA assay, and HPLC. The quantitative distribution of antibody-drug conjugates in terms of p may also be determined. In some instances, separation, purification, and characterization of homogeneous Antibody-drug-conjugates where p is a certain value from Antibody-drug-Conjugates with other drug loadings may be achieved by means such as reverse phase HPLC or electrophoresis. In exemplary embodiments, p is from 2 to 8.

[0116] Each of these units is described in more detail herein.

LINKER UNITS

[0165] Typically, the antibody-drug conjugate compounds comprise a linker region between the auristatin compound and the anti-CD30 antibody. A "Linker unit" (LU) is a bifunctional compound that can be used to link a Drug unit and an antibody unit to form an antibody-drug conjugate compound. In some embodiments, the linker

is cleavable under intracellular conditions, such that cleavage of the linker releases the auristatin compound from the antibody in the intracellular environment.

[0166] For example, in some embodiments, the linker is cleavable by a cleaving agent that is present in the intracellular environment (*e.g.*, within a lysosome or endosome or caveolea). The linker can be, *e.g.*, a peptidyl linker that is cleaved by an intracellular peptidase or protease enzyme, including, but not limited to, a lysosomal or endosomal protease. Typically, the peptidyl linker is at least two amino acids long or at least three amino acids long. Cleaving agents can include cathepsins B and D and plasmin, all of which are known to hydrolyze dipeptide drug derivatives resulting in the release of active drug inside target cells (*see*, *e.g.*, Dubowchik and Walker, 1999, *Pharm. Therapeutics* 83:67-123). Most typical are peptidyl linkers that are cleavable by enzymes that are present in CD30-expressing cells. For example, a peptidyl linker that is cleavable by the thiol-dependent protease cathepsin-B, which is highly expressed in cancerous tissue, can be used (*e.g.*, a Phe-Leu or a Gly-Phe-Leu-Gly linker). Other such linkers are described, *e.g.*, in U.S. Patent No. 6,214,345. In specific embodiments, the peptidyl linker cleavable by an intracellular protease is a Val-Cit linker or a Phe-Lys linker (*see, e.g.*, U.S. patent 6,214,345, which describes the synthesis of doxorubicin with the val-cit linker). One advantage of using intracellular proteolytic release of the therapeutic agent is that the agent is typically attenuated when conjugated and the serum stabilities of the conjugates are typically high.

[0167] In other embodiments, the cleavable linker is pH-sensitive, *i.e.*, sensitive to hydrolysis at certain pH values. Typically, the pH-senstive linker hydrolyzable under acidic conditions. For example, an acid-labile linker that is hydrolyzable in the lysosome (*e.g.*, a hydrazone, semicarbazone, thiosemicarbazone, cis-aconitic amide, orthoester, acetal, ketal, or the like) can be used. (*See, e.g.*, U.S. Patent Nos. 5,122,368; 5,824,805; 5,622,929; Dubowchik and Walker, 1999, *Pharm. Therapeutics* 83:67-123; Neville *et al.*, 1989, *Biol. Chem.* 264:14653-14661.) Such linkers are relatively stable under neutral pH conditions, such as those in the blood, but are unstable at below pH 5.5 or 5.0, the approximate pH of the lysosome. In certain embodiments, the hydrolyzable linker is a thioether linker (such as, *e.g.*, a thioether attached to the therapeutic agent via an acylhydrazone bond (*see, e.g.*, U.S. Patent No. 5,622,929).

[0168] In yet other embodiments, the linker is cleavable under reducing conditions (*e.g.*, a disulfide linker). A variety of disulfide linkers are known in the art, including, for example, those that can be formed using SATA (N-succinimidyl-S-acetylthioacetate), SPDP (N-succinimidyl-3-(2-pyridyldithio)propionate), SPDB (N-succinimidyl-3-(2-pyridyldithio)butyrate) and SMPT (N-succinimidyl-oxycarbonyl-alpha-methyl-alpha-(2-pyridyl-dithio)toluene), SPDB and SMPT. (*See, e.g.*, Thorpe *et al.*, 1987, *Cancer Res.* 47:5924-5931; Wawrzynczak *et al.*, In *Immunoconjugates: Antibody Conjugates in Radioimagery and Therapy of Cancer* (C. W. Vogel ed., Oxford U. Press, 1987. *See also* U.S. Patent No. 4,880,935.)

[0169] In yet other specific embodiments, the linker is a malonate linker (Johnson *et al.*, 1995, *Anticancer Res.* 15:1387-93), a maleimidobenzoyl linker (Lau *et al.*, 1995, *Bioorg-Med-Chem.* 3(10):1299-1304), or a 3'-N-amide analog (Lau *et al.*, 1995, *Bioorg-Med-Chem.* 3(10):1305-12).

[0170] In yet other embodiments, the linker unit is not cleavable and the drug is released by antibody degradation. (See U.S. Publication No. 20050238649 incorporated by reference herein in its entirety and for all purposes).

[0171] Typically, the linker is not substantially sensitive to the extracellular environment. As used herein, "not substantially sensitive to the extracellular environment," in the context of a linker, means that no more than about 20%, typically no more than about 15%, more typically no more than about 10%, and even more typically no more than about 5%, no more than about 3%, or no more than about 1% of the linkers, in a sample of antibody-drug conjugate compound, are cleaved when the antibody-drug conjugate compound presents in an extracellular environment (*e.g.*, in plasma). Whether a linker is not substantially sensitive to the extracellular environment can be determined, for example, by incubating with plasma the antibody-drug conjugate compound for a predetermined time period (*e.g.*, 2, 4, 8, 16, or 24 hours) and then quantitating the amount of free drug present in the plasma.

[0172] In other, non-mutually exclusive embodiments, the linker promotes cellular internalization. In certain embodiments, the linker promotes cellular internalization when conjugated to the therapeutic agent (*i.e.*, in the milieu of the linker-therapeutic agent moiety of the antibody-drug conjugate compound as described herein). In yet other embodiments, the linker promotes cellular internalization when conjugated to both the auristatin compound and the anti-CD30 antibody.

[0173] A variety of linkers that can be used with the present compositions and methods are described in, for example, WO 2004-010957, U.S. Publication No. 20060074008, U.S. Publication No. 20050238649, and U.S. Publication No. 20060024317 (each of which is incorporated by reference herein in its entirety and for all purposes).

[0174] In some embodiments, the Linker unit has the formula:

$$-A_a-W_w-Y_y-$$

wherein:-A- is a Stretcher unit,

a is 0 or 1,

each -W- is independently an Amino Acid unit,

w is an integer ranging from 0 to 12,

-Y- is a self-immolative Spacer unit, and

y is 0, 1 or 2.

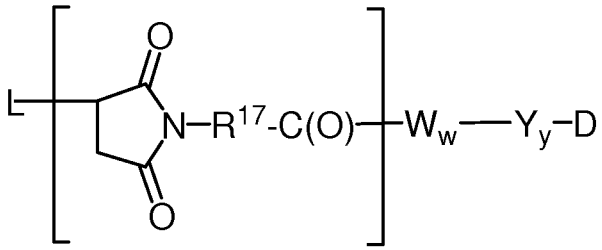
[0175] In some embodiments, a is 0 or 1, w is 0 or 1, and y is 0, 1 or 2. In some embodiments, if w is 1 to 12, then y is 1 or 2. In some embodiments, w is 2 to 12 and y is 1 or 2. In some embodiments, a is 0 or 1, w is 0 or 1, and y is 0 or 1. In some embodiments, p ranges from 1 to about 10, 1 to about 9, 1 to about 8, 1 to about 7, 1 to about 6, 1 to about 5, 1 to about 4, 1 to about 3, or 1 to 2. In some embodiments, p ranges from 2 to about 8, 2 to about 7, 2 to about 6, 2 to about 5, 2 to about 4 or 2 to 3. In other embodiments, p is 1, 2, 3, 4, 5 or 6. In some embodiments, p is 2 or 4.

THE STRETCHER UNIT

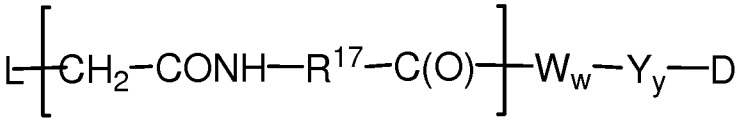
[0176] The Stretcher unit (A), when present, is capable of linking an antibody unit to an Amino Acid unit (-W-), if present, to a Spacer unit (-Y-), if present; or to a Drug unit (-D). Useful functional groups that can be present on an anti-CD30 antibody, either naturally or via chemical manipulation include, but are not limited to, sulfhydryl, amino, hydroxyl, the anomeric hydroxyl group of a carbohydrate, and carboxyl. Suitable functional groups are sulfhydryl and amino. Sulfhydryl groups can be generated by reduction of the intramolecular disulfide bonds of an anti-CD30 antibody. Alternatively, sulfhydryl groups can be generated by reaction of an amino group of a lysine moiety of an anti-CD30 antibody with 2-

iminothiolane (Traut's reagent) or other sulfhydryl generating reagents. In certain embodiments, the anti-CD30 antibody is a recombinant antibody and is engineered to carry one or more lysines. In certain other embodiments, the recombinant anti-CD30 antibody is engineered to carry additional sulfhydryl groups, *e.g.*, additional cysteines

[0177] In some embodiments, the Stretcher unit forms a bond with a sulfur atom of the antibody unit. The sulfur atom can be derived from a sulfhydryl group of an antibody. Representative Stretcher units of this embodiment are depicted within the square brackets of Formulas IIIa and IIIb, wherein L-, -W-, -Y-, -D, w and y are as defined above, and R_{17} is selected from -C_1-C_{10} alkylene-, -C_1-C_{10} alkenylene-, -C_1-C_{10} alkynylene-, -carbocyclo-, -O-(C_1-C_8 alkylene)-, O-(C_1-C_8 alkenylene)-, -O-(C_1-C_8 alkynylene)-, -arylene-, -C_1-C_{10} alkylene-arylene-, -C_2-C_{10} alkenylene-arylene, -C_2-C_{10} alkynylene-arylene, -arylene-C_1-C_{10} alkylene-, -arylene-C_2-C_{10} alkenylene-, -arylene-C_2-C_{10} alkynylene-, -C_1-C_{10} alkylene-(carbocyclo)-, -C_2-C_{10} alkenylene-(carbocyclo)-, -C_2-C_{10} alkynylene-(carbocyclo)-, -(carbocyclo)-C_1-C_{10} alkylene-, -(carbocyclo)-C_2-C_{10} alkenylene-, -(carbocyclo)-C_2-C_{10} alkynylene, heterocyclo-, -C_1-C_{10} alkylene-(heterocyclo)-, -C_2-C_{10} alkenylene-(heterocyclo)-, -C_2-C_{10} alkynylene-(heterocyclo)-, -(heterocyclo)-C_1-C_{10} alkylene-, -(heterocyclo)-C_2-C_{10} alkenylene-, -(heterocyclo)-C_1-C_{10} alkynylene-, -$(CH_2CH_2O)_r$-, or -$(CH_2CH_2O)_r$-CH_2-, and r is an integer ranging from 1-10, wherein said alkyl, alkenyl, alkynyl, alkylene, alkenylene, alkynyklene, aryl, carbocyle, carbocyclo, heterocyclo, and arylene radicals, whether alone or as part of another group, are optionally substituted. Alkylene, alkenylene, alkynylene radicals, whether alone or as part of another group, can be optionally substituted with, for example, one or more groups independently selected from A1; carbocyclo radicals, whether alone or as part of another group, can be optionally substituted with, for example, one or more groups independently selected from A2; arylene radicals, whether alone or as part of another group, can be optionally substituted with, for example, one or more groups independently selected from A3; heterocyclo radicals, whether alone or as part of another group, can be optionally substituted with, for example, one or more groups independently selected from A4. A1, A2, A3, and A4 are as defined herein. It is to be understood from all the exemplary embodiments that even where not denoted expressly, from 1 to 20 drug moieties can be linked to an antibody (p = 1-20).

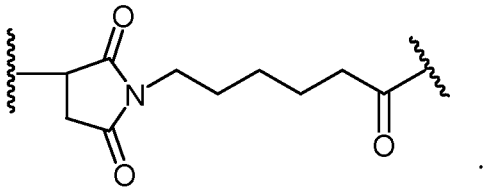


IIIa

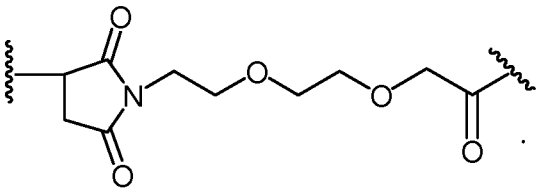


IIIb

[0178] An illustrative Stretcher unit is that of Formula IIIa wherein R^{17} is $-(CH_2)_5-$:

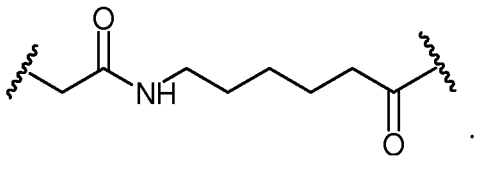


[0179] Another illustrative Stretcher unit is that of Formula IIIa wherein R^{17} is $-(CH_2CH_2O)_r-CH_2-$; and r is 2:

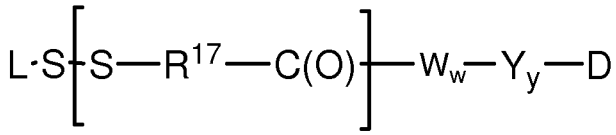


[0180] An illustrative Stretcher unit is that of Formula IIIa wherein R^{17} is -arylene- or arylene-C_1-C_{10} alkylene-. In some embodiments, the aryl group is an unsubstituted phenyl group.

[0181] Still another illustrative Stretcher unit is that of Formula IIIb wherein R^{17} is $-(CH_2)_5-$:

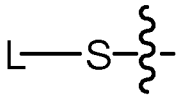


[0182] In certain embodiments, the Stretcher unit is linked to the antibody unit via a disulfide bond between a sulfur atom of the antibody unit and a sulfur atom of the Stretcher unit. A representative Stretcher unit of this embodiment is depicted within the square brackets of Formula IV, wherein R^{17}, L-, -W-, -Y-, -D, w and y are as defined above.

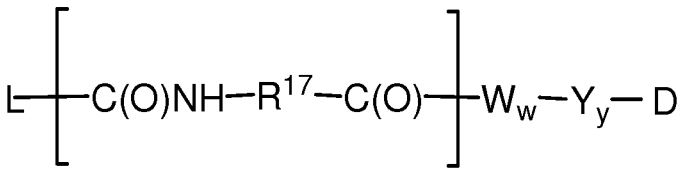


IV

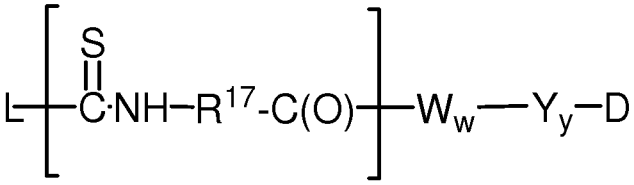
[0183] It should be noted that throughout this application, the S moiety in the formula below refers to a sulfur atom of the antibody unit, unless otherwise indicated by context.



[0184] In yet other embodiments, the Stretcher contains a reactive site that can form a bond with a primary or secondary amino group of an antibody. Examples of these reactive sites include, but are not limited to, activated esters such as succinimide esters, 4- nitrophenyl esters, pentafluorophenyl esters, tetrafluorophenyl esters, anhydrides, acid chlorides, sulfonyl chlorides, isocyanates and isothiocyanates. Representative Stretcher units of this embodiment are depicted within the square brackets of Formulas Va and Vb, wherein -R^{17}-, L-, -W-, -Y-, -D, w and y are as defined above;

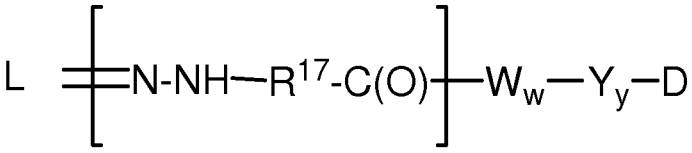


Va

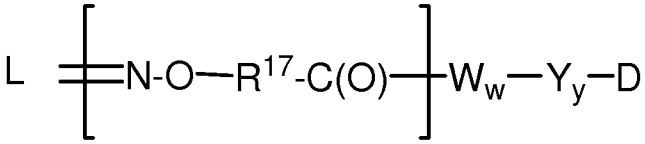


Vb

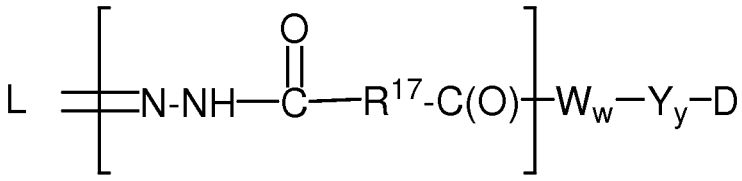
[0185] In some embodiments, the Stretcher contains a reactive site that is reactive to a modified carbohydrate's (-CHO) group that can be present on an antibody. For example, a carbohydrate can be mildly oxidized using a reagent such as sodium periodate and the resulting (-CHO) unit of the oxidized carbohydrate can be condensed with a Stretcher that contains a functionality such as a hydrazide, an oxime, a primary or secondary amine, a hydrazine, a thiosemicarbazone, a hydrazine carboxylate, and an arylhydrazide such as those described by Kaneko *et al.*, 1991, *Bioconjugate Chem.* 2:133-41. Representative Stretcher units of this embodiment are depicted within the square brackets of Formulas VIa, VIb, and VIc, wherein -R^{17}-, L-, -W-, -Y-, -D, w and y are as defined above.



VIa



VIb

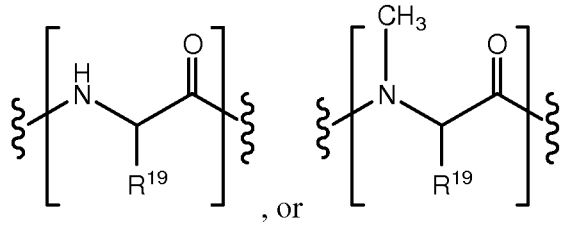


VIc

THE AMINO ACID UNIT

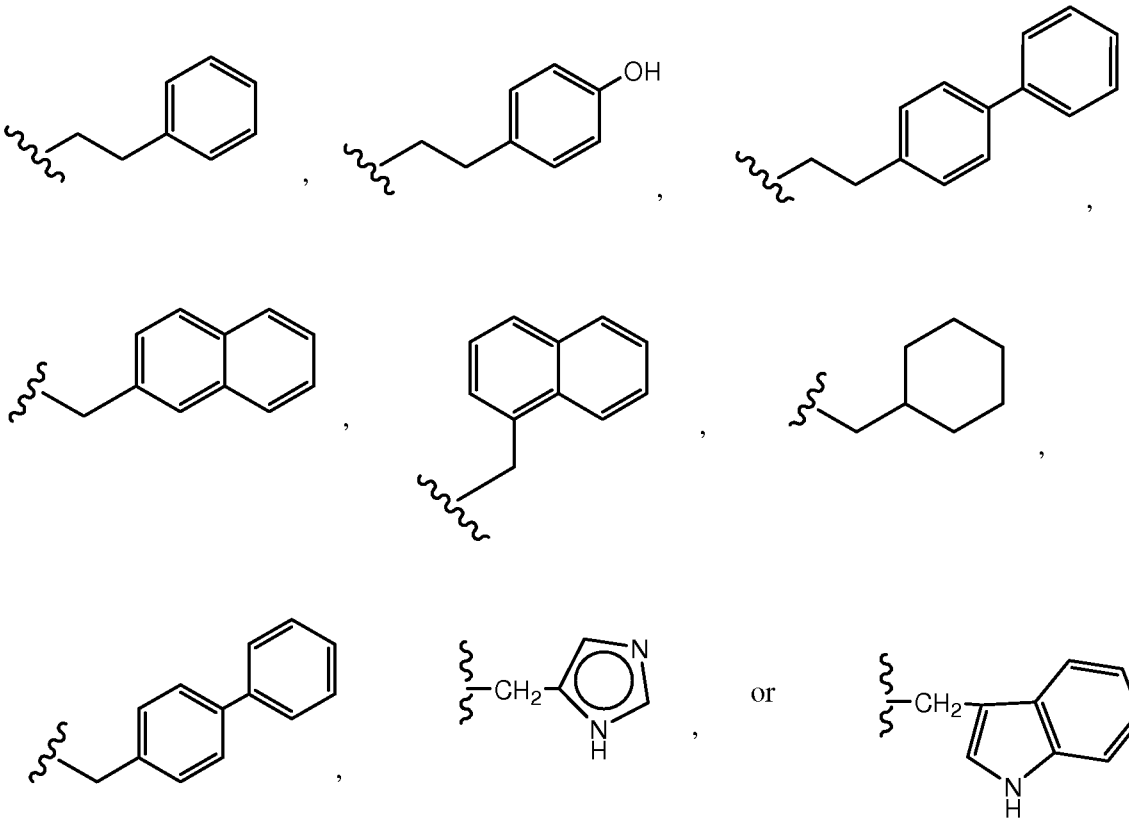
[0186] The Amino Acid unit (-W-), when present, links the Stretcher unit to the Spacer unit if the Spacer unit is present, links the Stretcher unit to the Drug moiety if the Spacer unit is absent, and links the antibody unit to the Drug unit if the Stretcher unit and Spacer unit are absent.

[0187] When present, W_w- is a monopeptide dipeptide, tripeptide, tetrapeptide, pentapeptide, hexapeptide, heptapeptide, octapeptide, nonapeptide, decapeptide, undecapeptide or dodecapeptide unit. Each -W- unit independently has the formula denoted below in the square brackets, and w is an integer ranging from 0 to 12:



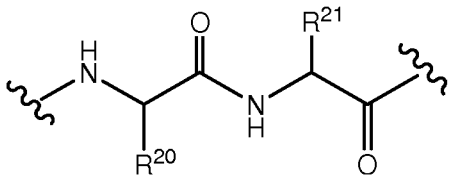
, or

wherein R^{19} is hydrogen, methyl, isopropyl, isobutyl, *sec*-butyl, benzyl, *p*-hydroxybenzyl, $-CH_2OH$, $-CH(OH)CH_3$, $-CH_2CH_2SCH_3$, $-CH_2CONH_2$, $-CH_2COOH$, $-CH_2CH_2CONH_2$, $-CH_2CH_2COOH$, $-(CH_2)_3NHC(=NH)NH_2$, $-(CH_2)_3NH_2$, $-(CH_2)_3NHCOCH_3$, $-(CH_2)_3NHCHO$, $-(CH_2)_4NHC(=NH)NH_2$, $-(CH_2)_4NH_2$, $-(CH_2)_4NHCOCH_3$, $-(CH_2)_4NHCHO$, $-(CH_2)_3NHCONH_2$, $-(CH_2)_4NHCONH_2$, $-CH_2CH_2CH(OH)CH_2NH_2$, 2-pyridylmethyl-, 3-pyridylmethyl-, 4-pyridylmethyl-, phenyl, cyclohexyl,



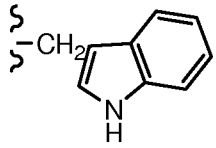
[0188] In some embodiments, the Amino Acid unit can be enzymatically cleaved by one or more enzymes, including a cancer or tumor-associated protease, to liberate the Drug unit (-D), which in one embodiment is protonated *in vivo* upon release to provide a Drug (D).

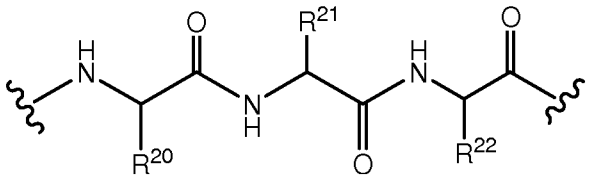
[0189] In certain embodiments, the Amino Acid unit can comprise natural amino acids. In other embodiments, the Amino Acid unit can comprise non-natural amino acids. Illustrative Ww units are represented by formulas (VII)-(IX):



(**VII**)

wherein R^{20} and R^{21} are as follows:

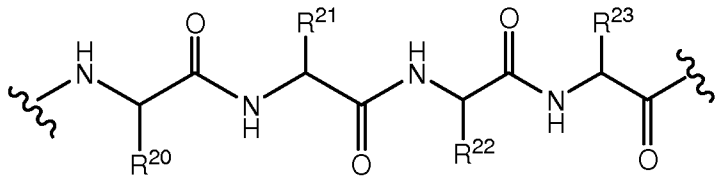
R^{20}	R^{21}
Benzyl	$(CH_2)_4NH_2$;
methyl	$(CH_2)_4NH_2$;
isopropyl	$(CH_2)_4NH_2$;
isopropyl	$(CH_2)_3NHCONH_2$;
benzyl	$(CH_2)_3NHCONH_2$;
isobutyl	$(CH_2)_3NHCONH_2$;
sec-butyl	$(CH_2)_3NHCONH_2$;
	$(CH_2)_3NHCONH_2$;
benzyl	methyl;
benzyl	$(CH_2)_3NHC(=NH)NH_2$;



(**VIII**)

wherein R^{20}, R^{21} and R^{22} are as follows:

R^{20}	R^{21}	R^{22}
benzyl	benzyl	$(CH_2)_4NH_2$;
isopropyl	benzyl	$(CH_2)_4NH_2$; and
H	benzyl	$(CH_2)_4NH_2$;



(IX)

wherein R^{20}, R^{21}, R^{22} and R^{23} are as follows:

R^{20}	R^{21}	R^{22}	R^{23}
H	benzyl	isobutyl	H; and
methyl	isobutyl	methyl	isobutyl.

[0190] Exemplary Amino Acid units include, but are not limited to, units of formula VII where: R^{20} is benzyl and R^{21} is $-(CH_2)_4NH_2$; R^{20} is isopropyl and R^{21} is $-(CH_2)_4NH_2$; or R^{20} is isopropyl and R^{21} is $-(CH_2)_3NHCONH_2$. Another exemplary Amino Acid unit is a unit of formula VIII wherein R^{20} is benzyl, R^{21} is benzyl, and R^{22} is $-(CH_2)_4NH_2$.

[0191] Useful $-W_w-$ units can be designed and optimized in their selectivity for enzymatic cleavage by a particular enzyme, for example, a tumor-associated protease. In one embodiment, a $-W_w-$ unit is that whose cleavage is catalyzed by cathepsin B, C and D, or a plasmin protease.

[0192] In one embodiment, $-W_w-$ is a dipeptide, tripeptide, tetrapeptide or pentapeptide. When R^{19}, R^{20}, R^{21}, R^{22} or R^{23} is other than hydrogen, the carbon atom to which R^{19}, R^{20}, R^{21}, R^{22} or R^{23} is attached is chiral.

[0193] Each carbon atom to which R^{19}, R^{20}, R^{21}, R^{22} or R^{23} is attached is independently in the (S) or (R) configuration.

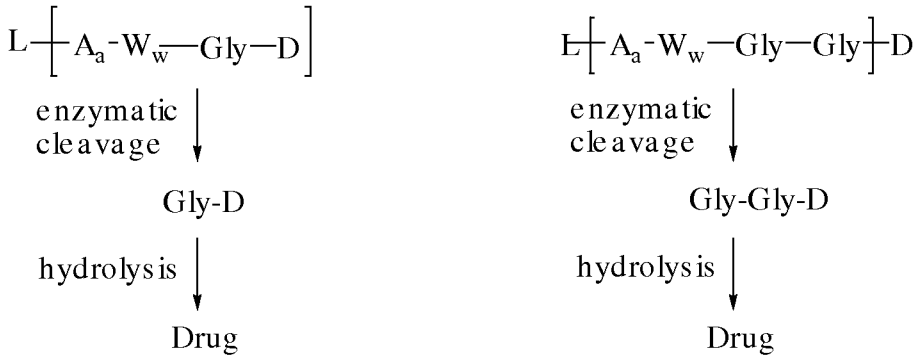
[0194] In one aspect of the Amino Acid unit, the Amino Acid unit is valine-citrulline (*i.e.,* vc or val-cit). In another aspect, the Amino Acid unit is phenylalanine-lysine (i.e., fk). In yet another aspect of the Amino Acid unit, the Amino Acid unit is N-methylvaline-citrulline. In yet another aspect, the Amino Acid unit is 5-aminovaleric acid, homo phenylalanine lysine, tetraisoquinolinecarboxylate lysine, cyclohexylalanine lysine, isonepecotic acid lysine, beta-alanine lysine, glycine serine valine glutamine and isonepecotic acid.

THE SPACER UNIT

[0195] The Spacer unit (-Y-), when present, links an Amino Acid unit to the Drug unit when an Amino Acid unit is present. Alternately, the Spacer unit links the Stretcher unit to the Drug unit when the Amino Acid unit is absent. The Spacer unit also links the Drug unit to the antibody unit when both the Amino Acid unit and Stretcher unit are absent.

[0196] Spacer units are of two general types: non self-immolative or self-immolative. A non self-immolative Spacer unit is one in which part or all of the Spacer unit remains bound to the Drug moiety after cleavage, particularly enzymatic, of an Amino Acid unit from the antibody drug conjugate. Examples of a non self-immolative Spacer unit include, but are not limited to a (glycine-glycine) Spacer unit and a glycine Spacer unit (both depicted in Scheme 1) (infra). When a conjugate containing a glycine-glycine Spacer unit or a glycine Spacer unit undergoes enzymatic cleavage via an enzyme (*e.g.*, a tumor-cell associated-protease, a cancer-cell-associated protease or a lymphocyte-associated protease), a glycine-glycine-Drug moiety or a glycine-Drug moiety is cleaved from L-Aa-Ww-. In one embodiment, an independent hydrolysis reaction takes place within the target cell, cleaving the glycine-Drug moiety bond and liberating the Drug.

Scheme 1



[0197] In some embodiments, a non self-immolative Spacer unit (-Y-) is -Gly-. In some embodiments, a non self-immolative Spacer unit (-Y-) is -Gly-Gly-.

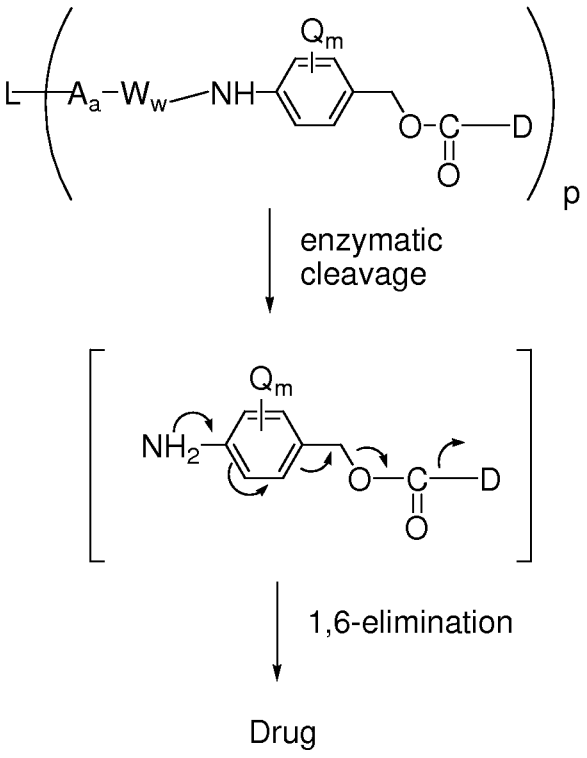
[0198] In one embodiment, a Drug-Linker conjugate is provided in which the Spacer unit is absent (y=0), or a pharmaceutically acceptable salt or solvate thereof.

[0199] Alternatively, a conjugate containing a self-immolative Spacer unit can release -D. As used herein, the term "self-immolative Spacer" refers to a bifunctional chemical moiety that is capable of covalently linking together two spaced chemical moieties into a stable tripartite molecule. It will spontaneously separate from the second chemical moiety if its bond to the first moiety is cleaved.

[0200] In some embodiments, $-Y_y-$ is a p-aminobenzyl alcohol (PAB) unit (see Schemes 2 and 3) whose phenylene portion is substituted with Q_m wherein Q is $-C_1-C_8$ alkyl, $-C_1-C_8$ alkenyl, $-C_1-C_8$ alkynyl, $-O-(C_1-C_8$ alkyl), $-O-(C_1-C_8$ alkenyl), $-O-(C_1-C_8$ alkynyl), -halogen,- nitro or -cyano; and m is an integer ranging from 0-4.

[0201] In some embodiments, -Y- is a PAB group that is linked to $-W_w-$ via the amino nitrogen atom of the PAB group, and connected directly to -D via a carbonate, carbamate or ether group. Without being bound by any particular theory or mechanism, Scheme 2 depicts a possible mechanism of Drug release of a PAB group which is attached directly to -D via a carbamate or carbonate group as described by Toki *et al.*, 2002, *J. Org. Chem.* 67:1866-1872.

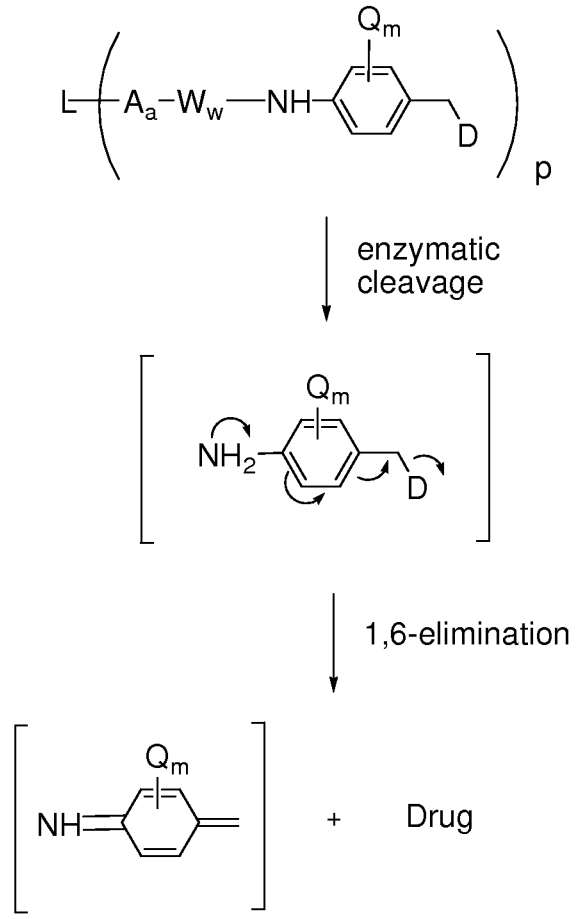
Scheme 2



[0202] In Scheme 2, Q is -C_1-C_8 alkyl, -C_1-C_8 alkenyl, -C_1-C_8 alkynyl, -O-(C_1-C_8 alkyl), -O-(C_1-C_8 alkenyl), -O-(C_1-C_8 alkynyl), -halogen, -nitro or -cyano; m is an integer ranging from 0-4; and p ranges from 1 to about 20.

[0203] Without being bound by any particular theory or mechanism, Scheme 3 depicts a possible mechanism of Drug release of a PAB group which is attached directly to -D via an ether or amine linkage, wherein D includes the oxygen or nitrogen group that is part of the Drug unit.

Scheme 3



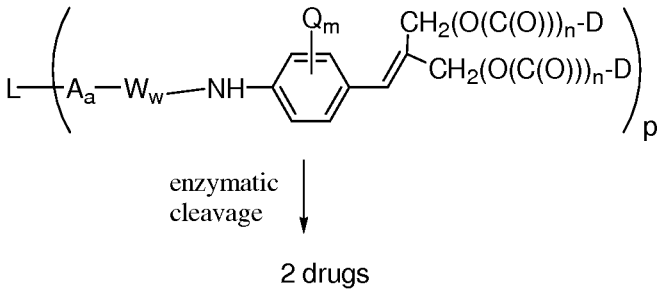
[0204] In Scheme 3, Q is -C_1-C_8 alkyl, -C_1-C_8 alkenyl, -C_1-C_8 alkynyl, -O-(C_1-C_8 alkyl), -O-(C_1-C_8 alkenyl), -O-(C_1-C_8 alkynyl), -halogen, -nitro or -cyano; m is an integer ranging from 0-4; and p ranges from 1 to about 20.

[0205] Other examples of self-immolative spacers include, but are not limited to, aromatic compounds that are electronically similar to the PAB group such as 2-aminoimidazol-5-methanol derivatives (Hay *et al.*, 1999, *Bioorg. Med. Chem. Lett.* 9:2237) and ortho or para-aminobenzylacetals. Spacers can be used that undergo

cyclization upon amide bond hydrolysis, such as substituted and unsubstituted 4-aminobutyric acid amides (Rodrigues *et al.*, 1995, *Chemistry Biology* 2:223), appropriately substituted bicyclo[2.2.1] and bicyclo[2.2.2] ring systems (Storm *et al.*, 1972, *J. Amer. Chem. Soc.* 94:5815) and 2-aminophenylpropionic acid amides (Amsberry *et al.*, 1990, *J. Org. Chem.* 55:5867). Elimination of amine-containing drugs that are substituted at the α-position of glycine (Kingsbury *et al.*, 1984, *J. Med. Chem.* 27:1447) are also examples of self-immolative spacers.

[0206] In one embodiment, the Spacer unit is a branched bis(hydroxymethyl)-styrene (BHMS) unit as depicted in Scheme 4, which can be used to incorporate and release multiple drugs.

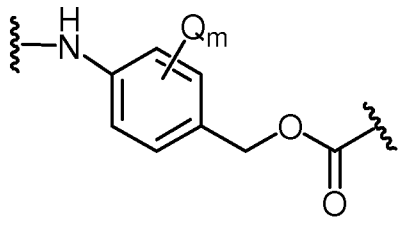
Scheme 4



[0207] In Scheme 4, Q is -C_1-C_8 alkyl, -C_1-C_8 alkenyl, -C_1-C_8 alkynyl, -O-(C_1-C_8 alkyl), -O-(C_1-C_8 alkenyl), -O-(C_1-C_8 alkynyl), -halogen, -nitro or -cyano; m is an integer ranging from 0-4; n is 0 or 1; and p ranges raging from 1 to about 20.

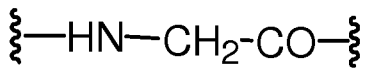
[0208] In some embodiments, the -D moieties are the same. In yet another embodiment, the -D moieties are different.

[0209] In one aspect, Spacer units (-Y_y-) are represented by Formulas (**X**)-(**XII**):



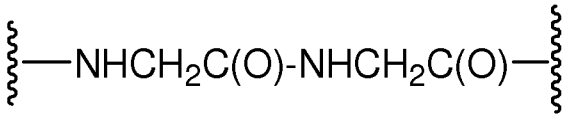
X

wherein Q is -C_1-C_8 alkyl, -C_1-C_8 alkenyl, -C_1-C_8 alkynyl, -O-(C_1-C_8 alkyl), -O-(C_1-C_8 alkenyl), -O-(C_1-C_8 alkynyl), -halogen, -nitro or -cyano; and m is an integer ranging from 0-4;



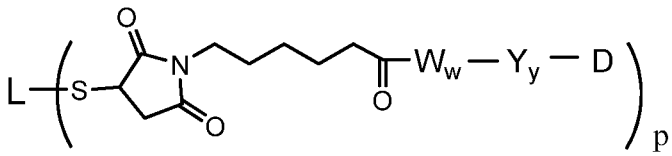
XI

and



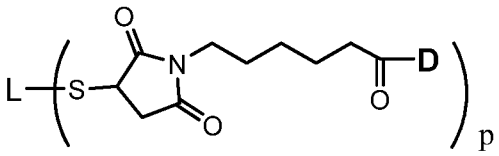
XII.

[0210] Embodiments of the Formula I and II comprising Antibody-drug conjugate compounds can include:

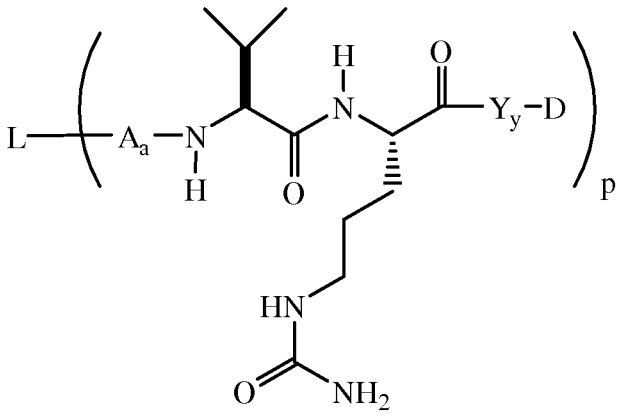


wherein w and y are each 0, 1 or 2,

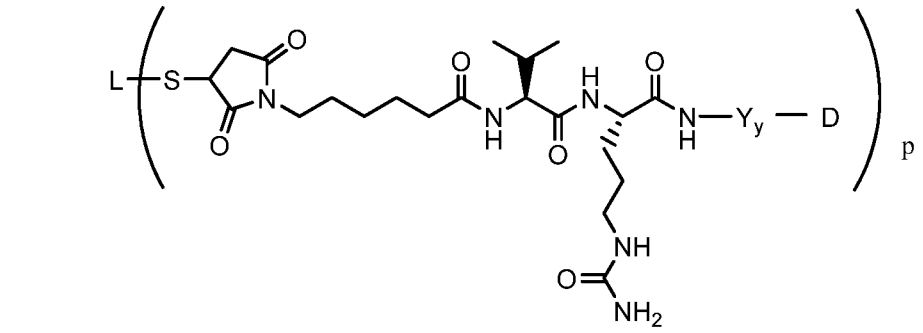
and,



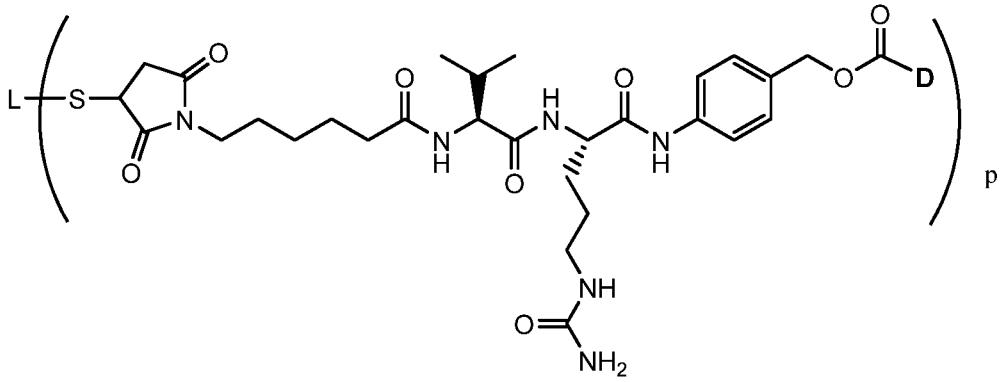
wherein w and y are each 0,



,



, and



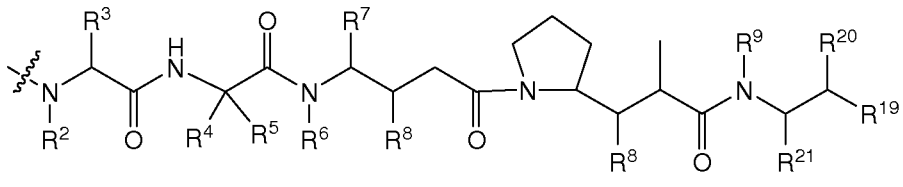
.

THE DRUG UNIT

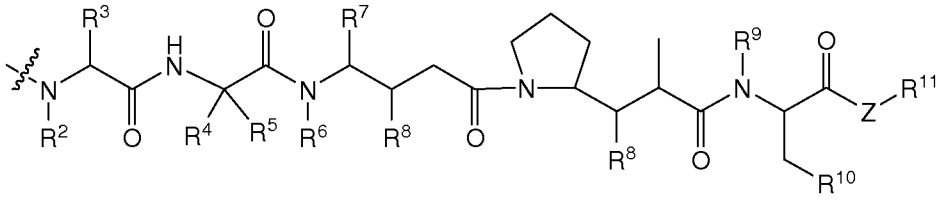
[0211] D is an auristatin drug compound having an atom that can form a bond with the Spacer unit, with the Amino Acid unit, with the Stretcher unit or with the antibody unit. In some embodiments, the Drug unit D has a N-terminal nitrogen atom that can form a bond with the Spacer unit. As used herein, the terms "Drug unit" and "Drug moiety" are synonymous and used interchangeably and refer to an auristatin drug unit or moeity.

[0212] In cetain preferred embodiments, the auristatin drug unit is auristatin E or a derivative thereof. Accordingly, the term "auristatin" as used herein is meant to include auristatin derivatives. The synthesis and structure of exemplary auristatin derivatives are described in U.S. Patent Application Publication Nos. 2003-0083263, 2005-0238649 and 2005-0009751; International Patent Publication No. WO 04/010957, International Patent Publication No. WO 02/088172, and U.S. Patent Nos. 6,323,315; 6,239,104; 6,034,065; 5,780,588; 5,665,860; 5,663,149; 5,635,483; 5,599,902; 5,554,725; 5,530,097; 5,521,284; 5,504,191; 5,410,024; 5,138,036; 5,076,973; 4,986,988; 4,978,744; 4,879,278; 4,816,444; and 4,486,414, each of which is incorporated by reference herein in its entirety.

[0213] In some embodiments, -D is an auristatin of the formula $\mathbf{D_E}$ or $\mathbf{D_F}$:



$\mathbf{D_E}$



$\mathbf{D_F}$

or a pharmaceutically acceptable salt or solvate form thereof;

wherein, independently at each location:

the wavy line indicates a bond;

$\mathbf{R^2}$ is -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, or -C_2-C_{20} alkynyl;

$\mathbf{R^3}$ is -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl, carbocycle, -C_1-C_{20} alkylene (carbocycle), -C_2-C_{20} alkenylene(carbocycle), -C_2-C_{20} alkynylene(carbocycle), -aryl, -C_1-C_{20} alkylene(aryl), -C_2-C_{20} alkenylene(aryl), -C_2-C_{20} alkynylene(aryl), -heterocycle, -C_1-C_{20} alkylene(heterocycle), -C_2-C_{20} alkenylene(heterocycle), or -C_2-C_{20} alkynylene(heterocycle);

$\mathbf{R^4}$ is -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl, carbocycle, -C_1-C_{20} alkylene (carbocycle), -C_2-C_{20} alkenylene(carbocycle), -C_2-C_{20} alkynylene(carbocycle), -aryl, -C_1-C_{20} alkylene(aryl), -C_2-C_{20} alkenylene(aryl), -C_2-C_{20} alkynylene(aryl), -heterocycle, -C_1-C_{20} alkylene(heterocycle), -C_2-C_{20} alkenylene(heterocycle), or -C_2-C_{20} alkynylene(heterocycle);

$\mathbf{R^5}$ is -H or -C_1-C_8 alkyl;

or $\mathbf{R^4}$ **and** $\mathbf{R^5}$ jointly form a carbocyclic ring and have the formula -$(CR^aR^b)_s$- wherein R^a and R^b are independently -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl, or carbocycle and s is 2, 3, 4, 5 or 6,

$\mathbf{R^6}$ is -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, or -C_2-C_{20} alkynyl;

$\mathbf{R^7}$ is -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl, -carbocycle, -C_1-C_{20} alkylene (carbocycle), -C_2-C_{20} alkenylene(carbocycle), -C_2-C_{20}

alkynylene(carbocycle), aryl, -C_1-C_{20} alkylene(aryl), -C_2-C_{20} alkenylene(aryl), -C_2-C_{20} alkynylene(aryl), heterocycle, -C_1-C_{20} alkylene(heterocycle), -C_2-C_{20} alkenylene(heterocycle), or -C_2-C_{20} alkynylene(heterocycle);

each $\mathbf{R^8}$ is independently -H, -OH, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl, -O-(C_1-C_{20} alkyl), -O-(C_2-C_{20} alkenyl), -O-(C_1-C_{20} alkynyl), or -carbocycle;

$\mathbf{R^9}$ is -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, or -C_2-C_{20} alkynyl;

$\mathbf{R^{19}}$ is -aryl, -heterocycle, or -carbocycle;

$\mathbf{R^{20}}$ is -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl, -carbocycle, -O-(C_1-C_{20} alkyl), -O-(C_2-C_{20} alkenyl), -O-(C_2-C_{20} alkynyl), or O$\mathbf{R^{18}}$ wherein $\mathbf{R^{18}}$ is -H, a hydroxyl protecting group, or a direct bond where O$\mathbf{R^{18}}$ represents =O;

$\mathbf{R^{21}}$ is -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, or -C_2-C_{20} alkynyl, -aryl, -heterocycle, or -carbocycle;

$\mathbf{R^{10}}$ is -aryl or -heterocycle;

Z is –O-, -S-, -NH-, or -NR^{12}-, wherein $\mathbf{R^{12}}$ is -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, or -C_2-C_{20} alkynyl;

$\mathbf{R^{11}}$ is -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl, -aryl, -heterocycle, -$(R^{13}O)_m$-R^{14}, or -$(R^{13}O)_m$-$CH(R^{15})_2$;

m is an integer ranging from 1-1000;

$\mathbf{R^{13}}$ is -C_2-C_{20} alkylene, -C_2-C_{20} alkenylene, or -C_2-C_{20} alkynylene;

$\mathbf{R^{14}}$ is -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, or -C_2-C_{20} alkynyl;

each occurrence of $\mathbf{R^{15}}$ is independently -H, -COOH, –$(CH_2)_n$-$N(R^{16})_2$, –$(CH_2)_n$-SO_3H, –$(CH_2)_n$-SO_3-C_1-C_{20} alkyl, –$(CH_2)_n$-SO_3-C_2-C_{20} alkenyl, or –$(CH_2)_n$-SO_3-C_2-C_{20} alkynyl;

each occurrence of $\mathbf{R^{16}}$ is independently -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl or –$(CH_2)_n$-COOH; and

n is an integer ranging from 0 to 6; wherein said alkyl, alkenyl, alkynyl, alkylene, alkenylene, alkynyklene, aryl, carbocyle, and heterocycle radicals, whether alone or as part of another group, are optionally substituted.

[0214] Auristatins of the formula $\mathbf{D_E}$ or $\mathbf{D_F}$ include those wherein

$\mathbf{R^2}$ is -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, or -C_2-C_{20} alkynyl, each of which is optionally substituted with one or more groups independently selected from A1;

$\mathbf{R^3}$ is -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl, monocyclic C_3-C_6 carbocycle, -C_1-C_{20} alkylene(monocyclic C_3-C_6 carbocycle), -C_2-C_{20}

alkenylene(monocyclic C_3-C_6 carbocycle), -C_2-C_{20} alkynylene(monocyclic C_3-C_6 carbocycle), -C_6-C_{10} aryl, -C_1-C_{20} alkylene(C_6-C_{10} aryl), -C_2-C_{20} alkenylene(C_6-C_{10} aryl), -C_2-C_{20} alkynylene(C_6-C_{10} aryl), -heterocycle, -C_1-C_{20} alkylene(heterocycle), -C_2-C_{20} alkenylene(heterocycle), or -C_2-C_{20} alkynylene(heterocycle); wherein said alkyl, alkenyl, alkynyl, alkylene, alkenylene, and alkynylene radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A1, said carbocycle radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A2, said aryl radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A3, and said heterocycle radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A4;

R^4 is -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl, monocyclic C_3-C_6 carbocycle, -C_1-C_{20} alkylene(monocyclic C_3-C_6 carbocycle), -C_2-C_{20} alkenylene(monocyclic C_3-C_6 carbocycle), -C_2-C_{20} alkynylene(monocyclic C_3-C_6 carbocycle), -C_6-C_{10} aryl, -C_1-C_{20} alkylene(C_6-C_{10} aryl), -C_2-C_{20} alkenylene(C_6-C_{10} aryl), -C_2-C_{20} alkynylene(C_6-C_{10} aryl), -heterocycle, -C_1-C_{20} alkylene(heterocycle), -C_2-C_{20} alkenylene(heterocycle), or -C_2-C_{20} alkynylene(heterocycle); wherein said alkyl, alkenyl, alkynyl, alkylene, alkenylene, and alkynylene radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A1, said carbocycle radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A2, said aryl radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A3, and said heterocycle radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A4;

R^5 is -H or -C_1-C_8 alkyl;

or **R^4 and R^5** jointly form a carbocyclic ring and have the formula -$(CR^aR^b)_s$- wherein R^a and R^b are independently -H, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, or carbocycle, and s is 2, 3, 4, 5 or 6;

R^6 is -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, or -C_2-C_{20} alkynyl, wherein said alkyl, alkenyl and alkynyl radicals are optionally substituted with one or more groups independently selected from A1;

$\mathbf{R^7}$ is -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl, monocyclic C_3-C_6 carbocycle, -C_1-C_{20} alkylene(monocyclic C_3-C_6 carbocycle), -C_2-C_{20} alkenylene(monocyclic C_3-C_6 carbocycle), -C_2-C_{20} alkynylene(monocyclic C_3-C_6 carbocycle), -C_6-C_{10} aryl, -C_1-C_{20} alkylene(C_6-C_{10} aryl), -C_2-C_{20} alkenylene(C_6-C_{10} aryl), -C_2-C_{20} alkynylene(C_6-C_{10} aryl), -heterocycle, -C_1-C_{20} alkylene(heterocycle), -C_2-C_{20} alkenylene(heterocycle), or -C_2-C_{20} alkynylene(heterocycle); wherein said alkyl, alkenyl, alkynyl, alkylene, alkenylene, and alkynylene radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A1, said carbocycle radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A2, said aryl radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A3, and said heterocycle radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A4;

each $\mathbf{R^8}$ is independently -H, -OH, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl, -O-(C_1-C_{20} alkyl), -O-(C_2-C_{20} alkenyl), -O-(C_1-C_{20} alkynyl), or carbocycle, wherein said alkyl, alkenyl, and alkynyl radicals, whether alone or as part of another group, are optionally substituted with one or more groups independently selected from A1 and said carbocycle is optionally substituted with one or more groups independently selected from A2;

$\mathbf{R^9}$ is -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, or -C_2-C_{20} alkynyl; wherein said alkyl, alkenyl and alkynyl radical are optionally substituted with one or more groups independently selected from A1;

$\mathbf{R^{19}}$ is -aryl, -heterocycle, or -carbocycle; wherein said carbocycle radical is optionally substituted with one or more groups independently selected from A2, said aryl radical is optionally substituted with one or more groups independently selected from A3, and said heterocycle radical is optionally substituted with one or more groups independently selected from A4;

$\mathbf{R^{20}}$ is selected from -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl, -carbocycle, -OH, -O-(C_1-C_{20} alkyl), -O-(C_2-C_{20} alkenyl), -O-(C_2-C_{20} alkynyl)or OR^{18}; wherein said alkyl, alkenyl, and alkynyl radicals, whether alone or as part of another group, are optionally substituted with one or more groups independently selected from A1, and said carbocycle is optionally substituted with one or more groups independently selected from A2;

$\mathbf{R^{18}}$ is -H, a hydroxyl protecting group, or a direct bond where OR^{18} represents =O;

$\mathbf{R^{21}}$ is selected from -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl, or -carbocycle; wherein said alkyl, alkenyl, and alkynyl radicals are optionally substituted with one or more groups independently selected from A1, and said carbocycle radical is optionally substituted with one or more groups independently selected from A2;

$\mathbf{R^{10}}$ is aryl optionally substituted with one or more groups independently selected from A3, or heterocycle optionally substituted with one or more groups independently selected from A4;

Z is –O-, -S-, -NH-, or -NR^{12}, wherein $\mathbf{R^{12}}$ is -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, or -C_2-C_{20} alkynyl, each of which is optionally substituted with one or more groups independently selected from A1;

$\mathbf{R^{11}}$ is -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl, -aryl, -heterocycle, -$(R^{13}O)_m$-R^{14}, or -$(R^{13}O)_m$-$CH(R^{15})_2$ wherein said alkyl, alkenyl and alkynyl radicals are optionally substituted with one or more groups independently selected from A1, said aryl radical is optionally substituted with one or more groups independently selected from A3, and said heterocycle is optionally substituted with one or more groups independently selected from A4;

m is an integer ranging from 1-1000;

$\mathbf{R^{13}}$ is -C_2-C_{20} alkylene, -C_2-C_{20} alkenylene, or -C_2-C_{20} alkynylene, each of which is optionally substituted with one or more groups independently selected from A1;

$\mathbf{R^{14}}$ is -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, or -C_2-C_{20} alkynyl wherein said alkyl, alkenyl and alkynyl radicals are optionally substituted with one or more groups independently selected from A1;

each occurrence of $\mathbf{R^{15}}$ is independently -H, -COOH, $-(CH_2)_n$-$N(R^{16})_2$, $-(CH_2)_n$-SO_3H, $-(CH_2)_n$-SO_3-C_1-C_{20} alkyl, $-(CH_2)_n$-SO_3-C_2-C_{20} alkenyl, or $-(CH_2)_n$-SO_3-C_2-C_{20} alkynyl wherein said alkyl, alkenyl and alkynyl radicals are optionally substituted with one or more groups independently selected from A1;

each occurrence of $\mathbf{R^{16}}$ is independently -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl or $-(CH_2)_n$-COOH wherein said alkyl, alkenyl and alkynyl radicals are optionally substituted with one or more groups independently selected from A1;

n is an integer ranging from 0 to 6;

A1 is halogen, -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2 , -C(O)NHR', -C(O)N(R')$_2$ -NHC(O)R', -SR', -SO_3R', -S(O)$_2$R', -S(O)R', -OH, =O, -N_3 , -NH_2, -NH(R'), -N(R')$_2$ and -CN, where each R' is independently selected from -H, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, or -aryl, and wherein said -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -aryl, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, and -C_2-C_8 alkynyl groups can be optionally further substituted with one or more groups independently selected from -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, -halogen, -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -aryl, -C(O)R'', -OC(O)R'', -C(O)OR'', -C(O)NH_2 , -C(O)NHR'', -C(O)N(R'')$_2$, -NHC(O)R'', -SR'', -SO_3R'', -S(O)$_2$R'', -S(O)R'', -OH, -N_3 , -NH_2, -NH(R''), -N(R'')$_2$ and -CN, where each R'' is independently selected from -H, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, or -aryl;

A2 is -halogen, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2 , -C(O)NHR', -C(O)N(R')$_2$ -NHC(O)R', -SR', -SO_3R', -S(O)$_2$R', -S(O)R', -OH, =O, -N_3 , -NH_2, -NH(R'), -N(R')$_2$ and -CN, where each R' is independently selected from -H, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, or -aryl and wherein said -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), and -aryl groups can be further optionally substituted with one or more substituents independently selected from -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, -halogen, -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -aryl, -C(O)R'', -OC(O)R'', -C(O)OR'', -C(O)NH_2 , -C(O)NHR'', -C(O)N(R'')$_2$, -NHC(O)R'', -SR'', -SO_3R'', -S(O)$_2$R'', -S(O)R'', -OH, -N_3 , -NH_2, -NH(R''), -N(R'')$_2$ and -CN, where each R'' is independently selected from -H, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, or -aryl;

A3 is -halogen, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2 , -C(O)NHR', -C(O)N(R')$_2$ -NHC(O)R', -SR', -SO_3R', -S(O)$_2$R', -S(O)R', -OH, -NO_2, -N_3 , -NH_2, -NH(R'), -N(R')$_2$ and -CN, where each R' is independently selected from -H, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, or -aryl and wherein said -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), and -aryl groups can be further optionally

substituted with one or more substituents independently selected from -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, -halogen, -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -aryl, -C(O)R'', -OC(O)R'', -C(O)OR'', -C(O)NH_2 , -C(O)NHR'', -C(O)N(R'')$_2$, -NHC(O)R'', -SR'', -SO_3R'', -S(O)$_2$R'', -S(O)R'', -OH, -N_3 , -NH_2, -NH(R''), -N(R'')$_2$ and -CN, where each R'' is independently selected from -H, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, or aryl;

and A4 is -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, -halogen, -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2 , -C(O)NHR', -C(O)N(R')$_2$ -NHC(O)R', -SR', -SO_3R', -S(O)$_2$R', -S(O)R', -OH, -N_3 , -NH_2, -NH(R'), -N(R')$_2$ and -CN, where each R' is independently selected from -H, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, or -aryl and wherein said -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, and aryl groups can be further optionally substituted with one or more substituents independently selected from -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, -halogen, -O-(C_1-C_8 alkyl), -O-(C_2-C_8 alkenyl), -O-(C_2-C_8 alkynyl), -aryl, -C(O)R'', -OC(O)R'', -C(O)OR'', -C(O)NH_2 , -C(O)NHR'', -C(O)N(R'')$_2$, -NHC(O)R'', -SR'', -SO_3R'', -S(O)$_2$R'', -S(O)R'', -OH, -N_3 , -NH_2, -NH(R''), -N(R'')$_2$ and -CN, where each R'' is independently selected from -H, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, or aryl; or a pharmaceutically acceptable salt or solvate form thereof.

[0215] Auristatins of the formula $\mathbf{D_E}$ include those wherein said alkyl, alkenyl, alkynyl, alkylene, alkenylene, alkynyklene, aryl, carbocyle, and heterocycle radicals are unsubstituted.

[0216] Auristatins of the formula $\mathbf{D_E}$ include those wherein the groups of R^2, R^3, R^4, R^5, R^6, R^7, R^8, and R^9 are unsubstituted and the groups of R^{19}, R^{20} and R^{21} are optionally substituted as described herein.

[0217] Auristatins of the formula $\mathbf{D_E}$ include those wherein

$\mathbf{R^2}$ is C_1-C_{20} alkyl optionally substituted with one or more groups independently selected from A1;

$\mathbf{R^3}$ and $\mathbf{R^7}$ are independently selected from -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl, monocyclic C_3-C_6 carbocycle, -C_1-C_{20} alkylene(monocyclic C_3-C_6 carbocycle), -C_2-C_{20} alkenylene(monocyclic C_3-C_6 carbocycle), -C_2-C_{20} alkynylene(monocyclic C_3-C_6 carbocycle), -C_6-C_{10} aryl, -C_1-C_{20} alkylene(C_6-C_{10} aryl), -C_2-C_{20} alkenylene(C_6-C_{10} aryl), -C_2-C_{20} alkynylene(C_6-C_{10} aryl), -heterocycle,

-C_1-C_{20} alkylene(heterocycle), -C_2-C_{20} alkenylene(heterocycle), or -C_2-C_{20} alkynylene(heterocycle); wherein said alkyl, alkenyl, alkynyl, alkylene, alkenylene, and alkynylene radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A1, said carbocycle radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A2, said aryl radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A3, and said heterocycle radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A4;

R^4 is -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl, monocyclic C_3-C_6 carbocycle, -C_1-C_{20} alkylene(monocyclic C_3-C_6 carbocycle), -C_2-C_{20} alkenylene(monocyclic C_3-C_6 carbocycle), -C_2-C_{20} alkynylene(monocyclic C_3-C_6 carbocycle), -C_6-C_{10} aryl, -C_1-C_{20} alkylene(C_6-C_{10} aryl), -C_2-C_{20} alkenylene(C_6-C_{10} aryl), -C_2-C_{20} alkynylene(C_6-C_{10} aryl), -heterocycle, -C_1-C_{20} alkylene(heterocycle), -C_2-C_{20} alkenylene(heterocycle), or -C_2-C_{20} alkynylene(heterocycle); wherein said alkyl, alkenyl, alkynyl, alkylene, alkenylene, and alkynylene radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A1, said carbocycle radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A2, said aryl radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A3, and said heterocycle radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A4;

R^5 is -H or -C_1-C_8 alkyl;

or **R^4 and R^5** jointly form a carbocyclic ring and have the formula -$(CR^aR^b)_s$- wherein R^a and R^b are independently selected from -H, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, or carbocycle, and s is selected from 2, 3, 4, 5 or 6;

R^6 is -C_1-C_{20} alkyl optionally substituted with one or more groups independently selected from A1;

each **R^8** is independently selected from -OH, -O-(C_1-C_{20} alkyl), -O-(C_2-C_{20} alkenyl), or -O-(C_2-C_{20} alkynyl) wherein said alkyl, alkenyl, and alkynyl radicals are optionally substituted with one or more groups independently selected from A1;

$\mathbf{R^9}$ is -hydrogen or -C_1-C_{20} alkyl optionally substituted with one or more groups independently selected from A1;

$\mathbf{R^{19}}$ is aryl, heterocycle, or carbocycle; wherein said carbocycle radical is optionally substituted with one or more groups independently selected from A2, said aryl radical is optionally substituted with one or more groups independently selected from A3, and said heterocycle radical is optionally substituted with one or more groups independently selected from A4;

$\mathbf{R^{20}}$ is $\mathbf{OR^{18}}$; wherein $\mathbf{R^{18}}$ is -H, a hydroxyl protecting group, or a direct bond where OR^{18} represents =O;

$\mathbf{R^{21}}$ is selected from -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl, or -carbocycle; wherein said alkyl, alkenyl, and alkynyl radicals are optionally substituted with one or more groups independently selected from A1, and said carbocycle radical is optionally substituted with one or more groups independently selected from A2;

and A1, A2, A3, and A4 are as defined herein; or a pharmaceutically acceptable salt or solvate form thereof.

[0218] Auristatins of the formula $\mathbf{D_E}$ include those wherein

$\mathbf{R^2}$ is -C_1-C_8 alkyl;

$\mathbf{R^3}$, $\mathbf{R^4}$ and $\mathbf{R^7}$ are independently selected from -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl, monocyclic C_3-C_6 carbocycle, -C_1-C_{20} alkylene(monocyclic C_3-C_6 carbocycle), -C_2-C_{20} alkenylene(monocyclic C_3-C_6 carbocycle), -C_2-C_{20} alkynylene(monocyclic C_3-C_6 carbocycle), -C_6-C_{10} aryl, -C_1-C_{20} alkylene(C_6-C_{10} aryl), -C_2-C_{20} alkenylene(C_6-C_{10} aryl), -C_2-C_{20} alkynylene(C_6-C_{10} aryl), -heterocycle, -C_1-C_{20} alkylene(heterocycle), -C_2-C_{20} alkenylene(heterocycle), or -C_2-C_{20} alkynylene(heterocycle); wherein said alkyl, alkenyl, alkynyl, alkylene, alkenylene, and alkynylene radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A1, said carbocycle radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A2, said aryl radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A3, and said heterocycle radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A4;

$\mathbf{R^5}$ is -hydrogen;

$\mathbf{R^6}$ is -C_1-C_8 alkyl;

each $\mathbf{R^8}$ is independently selected from -OH, -O-(C_1-C_{20} alkyl), -O-(C_2-C_{20} alkenyl), or -O-(C_2-C_{20} alkynyl) wherein said alkyl, alkenyl, and alkynyl radicals are optionally substituted with one or more groups independently selected from A1;

$\mathbf{R^9}$ is -hydrogen or -C_1-C_8 alkyl;

$\mathbf{R^{19}}$ is phenyl optionally substituted with one or more groups independently selected from A3;

$\mathbf{R^{20}}$ is $\mathbf{OR^{18}}$; wherein $\mathbf{R^{18}}$ is H, a hydroxyl protecting group, or a direct bond where $\mathbf{OR^{18}}$ represents =O;

$\mathbf{R^{21}}$ is selected from -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl, or -carbocycle; wherein said alkyl, alkenyl, and alkynyl radicals are optionally substituted with one or more groups independently selected from A1, and said carbocycle radical is optionally substituted with one or more groups independently selected from A2; and

A1, A2, A3, and A4 are as defined herein; or a pharmaceutically acceptable salt or solvate form thereof.

[0219] Auristatins of the formula $\mathbf{D_E}$ include those wherein

$\mathbf{R^2}$ is methyl;

$\mathbf{R^3}$ is -H, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, or -C_2-C_8 alkynyl, wherein said alkyl, alkenyl and alkynyl radicals are optionally optionally substituted with one or more groups independently selected from A1;

$\mathbf{R^4}$ is -H, -C_1-C_8 alkyl, -C_2-C_8 alkenyl, -C_2-C_8 alkynyl, monocyclic C_3-C_6 carbocycle, -C_6-C_{10} aryl, -C_1-C_8 alkylene(C_6-C_{10} aryl), -C_2-C_8 alkenylene(C_6-C_{10} aryl), -C_2-C_8 alkynylene(C_6-C_{10} aryl), -C_1-C_8 alkylene (monocyclic C_3-C_6 carbocycle), -C_2-C_8 alkenylene (monocyclic C_3-C_6 carbocycle), -C_2-C_8 alkynylene(monocyclic C_3-C_6 carbocycle); wherein said alkyl, alkenyl, alkynyl, alkylene, alkenylene, and alkynylene radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A1; said carbocyle radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A2; and said aryl radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A3;

$\mathbf{R^5}$ is H; $\mathbf{R^6}$ is methyl;

$\mathbf{R^7}$ is -C_1-C_8 alkyl, -C_2-C_8 alkenyl or -C_2-C_8 alkynyl;

each $\mathbf{R^8}$ is methoxy;

$\mathbf{R^9}$ is -hydrogen or -C_1-C_8 alkyl;

$\mathbf{R^{19}}$ is phenyl;

$\mathbf{R^{20}}$ is O$\mathbf{R^{18}}$; wherein $\mathbf{R^{18}}$ is -H, a hydroxyl protecting group, or a direct bond where O$\mathbf{R^{18}}$ represents =O;

$\mathbf{R^{21}}$ is methyl; and A1, A2, and A3 are as defined herein; or a pharmaceutically acceptable salt or solvate form thereof.

[0220] Auristatins of the formula $\mathbf{D_E}$ include those wherein $\mathbf{R^2}$ is methyl; $\mathbf{R^3}$ is H or C_1-C_3 alkyl; $\mathbf{R^4}$ is C_1-C_5 alkyl; $\mathbf{R^5}$ is H; $\mathbf{R^6}$ is methyl; $\mathbf{R^7}$ is isopropyl or sec-butyl; $\mathbf{R^8}$ is methoxy; $\mathbf{R^9}$ is hydrogen or C_1-C_8 alkyl; $\mathbf{R^{19}}$ is phenyl; $\mathbf{R^{20}}$ is O$\mathbf{R^{18}}$; wherein $\mathbf{R^{18}}$ is H, a hydroxyl protecting group, or a direct bond where OR^{18} represents =O; and $\mathbf{R^{21}}$ is methyl; or a pharmaceutically acceptable salt or solvate form thereof.

[0221] Auristatins of the formula $\mathbf{D_E}$ include those wherein $\mathbf{R^2}$ is methyl or or C_1-C_3 alkyl; $\mathbf{R^3}$ is H or C_1-C_3 alkyl; $\mathbf{R^4}$ is C_1-C_5 alkyl; $\mathbf{R^5}$ is H; $\mathbf{R^6}$ is C_1-C_3 alkyl; $\mathbf{R^7}$ is C_1-C_5 alkyl; $\mathbf{R^8}$ is C_1-C_3 alkoxy; $\mathbf{R^9}$ is hydrogen or C_1-C_8 alkyl; $\mathbf{R^{19}}$ is phenyl; $\mathbf{R^{20}}$ is O$\mathbf{R^{18}}$; wherein $\mathbf{R^{18}}$ is H, a hydroxyl protecting group, or a direct bond where OR^{18} represents =O; and $\mathbf{R^{21}}$ is C_1-C_3 alkyl; or a pharmaceutically acceptable salt or solvate form thereof.

[0222] Auristatins of the formula $\mathbf{D_F}$ include those wherein

$\mathbf{R^2}$ is -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, or -C_2-C_{20} alkynyl, each of which is optionally substituted with one or more groups independently selected from A1;

$\mathbf{R^3}$, $\mathbf{R^4}$, and $\mathbf{R^7}$ are independently selected from -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl, monocyclic C_3-C_6 carbocycle, -C_1-C_{20} alkylene(monocyclic C_3-C_6 carbocycle), -C_2-C_{20} alkenylene(monocyclic C_3-C_6 carbocycle), -C_2-C_{20} alkynylene(monocyclic C_3-C_6 carbocycle), C_6-C_{10} aryl, -C_1-C_{20} alkylene(C_6-C_{10} aryl), -C_2-C_{20} alkenylene(C_6-C_{10} aryl), -C_2-C_{20} alkynylene(C_6-C_{10} aryl), -heterocycle, -C_1-C_{20} alkylene(heterocycle), -C_2-C_{20} alkenylene(heterocycle), or -C_2-C_{20} alkynylene(heterocycle); wherein said alkyl, alkenyl, alkynyl, alkylene, alkenylene, and alkynylene radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A1, said carbocycle radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A2, said aryl radicals whether alone or as part of another group are optionally substituted with one or more groups

independently selected from A3, and said heterocycle radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A4;

$\mathbf{R^5}$ is -H;

$\mathbf{R^6}$ is -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, or -C_2-C_{20} alkynyl, wherein said alkyl, alkenyl and alkynyl radicals are optionally substituted with one or more groups independently selected from A1;

each $\mathbf{R^8}$ is independently selected from -H, -OH, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl, -O-(C_1-C_{20} alkyl), -O-(C_2-C_{20} alkenyl), -O-(C_1-C_{20} alkynyl), or carbocycle, wherein said alkyl, alkenyl, and alkynyl radicals, whether alone or as part of another group, are optionally substituted with one or more groups independently selected from A1 and said carbocycle is optionally substituted with one or more groups independently selected from A2;

$\mathbf{R^9}$ is -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, or -C_2-C_{20} alkynyl; wherein said alkyl, alkenyl and alkynyl radical are optionally substituted with one or more groups independently selected from A1;

$\mathbf{R^{10}}$ is phenyl optionally substituted with one or more groups independently selected from A3;

Z is –O-, -S-, -NH-, or -NR^{12}, wherein $\mathbf{R^{12}}$ is -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, or -C_2-C_{20} alkynyl, each of which is optionally substituted with one or more groups independently selected from A1;

$\mathbf{R^{11}}$ is -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl, -aryl, -heterocycle, -$(R^{13}O)_m$-R^{14}, or -$(R^{13}O)_m$-$CH(R^{15})_2$ wherein said alkyl, alkenyl and alkynyl radicals are optionally substituted with one or more groups independently selected from A1, said aryl radical is optionally substituted with one or more groups independently selected from A3, and said heterocycle is optionally substituted with one or more groups independently selected from A4;

m is an integer ranging from 1-1000;

$\mathbf{R^{13}}$ is -C_2-C_{20} alkylene, -C_2-C_{20} alkenylene, or -C_2-C_{20} alkynylene, each of which is optionally substituted with one or more groups independently selected from A1;

$\mathbf{R^{14}}$ is -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, or -C_2-C_{20} alkynyl wherein said alkyl, alkenyl and alkynyl radicals are optionally substituted with one or more groups independently selected from A1;

each occurrence of $\mathbf{R^{15}}$ is independently -H, -COOH, $-(CH_2)_n-N(R^{16})_2$, $-(CH_2)_n-SO_3H$, $-(CH_2)_n-SO_3-C_1-C_{20}$ alkyl, $-(CH_2)_n-SO_3-C_2-C_{20}$ alkenyl, or $-(CH_2)_n-SO_3-C_2-C_{20}$ alkynyl wherein said alkyl, alkenyl and alkynyl radicals are optionally substituted with one or more groups independently selected from A1;

each occurrence of $\mathbf{R^{16}}$ is independently -H, $-C_1-C_{20}$ alkyl, $-C_2-C_{20}$ alkenyl, $-C_2-C_{20}$ alkynyl or $-(CH_2)_n-COOH$ wherein said alkyl, alkenyl and alkynyl radicals are optionally substituted with one or more groups independently selected from A1;

n is an integer ranging from 0 to 6; and A1, A2, A3, and A4 are as defined herein; or a pharmaceutically acceptable salt or solvate form thereof.

[0223] Auristatins of the formula $\mathbf{D_F}$ include those wherein

$\mathbf{R^2}$ is methyl;

$\mathbf{R^3}$, $\mathbf{R^4}$, and $\mathbf{R^7}$ are independently selected from -H, $-C_1-C_{20}$ alkyl, $-C_2-C_{20}$ alkenyl, $-C_2-C_{20}$ alkynyl, monocyclic C_3-C_6 carbocycle, $-C_1-C_{20}$ alkylene(monocyclic C_3-C_6 carbocycle), $-C_2-C_{20}$ alkenylene(monocyclic C_3-C_6 carbocycle), $-C_2-C_{20}$ alkynylene(monocyclic C_3-C_6 carbocycle), $-C_6-C_{10}$ aryl, $-C_1-C_{20}$ alkylene(C_6-C_{10} aryl), $-C_2-C_{20}$ alkenylene(C_6-C_{10} aryl), $-C_2-C_{20}$ alkynylene(C_6-C_{10} aryl), -heterocycle, $-C_1-C_{20}$ alkylene(heterocycle), $-C_2-C_{20}$ alkenylene(heterocycle), or $-C_2-C_{20}$ alkynylene(heterocycle); wherein said alkyl, alkenyl, alkynyl, alkylene, alkenylene, and alkynylene radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A1, said carbocycle radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A2, said aryl radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A3, and said heterocycle radicals whether alone or as part of another group are optionally substituted with one or more groups independently selected from A4;

$\mathbf{R^5}$ is -H;

$\mathbf{R^6}$ is methyl;

each $\mathbf{R^8}$ is methoxy;

$\mathbf{R^9}$ is -H, $-C_1-C_{20}$ alkyl, $-C_2-C_{20}$ alkenyl, or $-C_2-C_{20}$ alkynyl; wherein said alkyl, alkenyl and alkynyl radical are optionally substituted with one or more groups independently selected from A1;

R^{10} is aryl optionally substituted with one or more groups independently selected from A3, or heterocycle optionally substituted with one or more groups independently selected from A4;

Z is –O-, -S-, -NH-, or -NR^{12}-, wherein **R^{12}** is -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, or -C_2-C_{20} alkynyl, each of which is optionally substituted with one or more groups independently selected from A1;

R^{11} is -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl, -aryl, -heterocycle, -$(R^{13}O)_m$-R^{14}, or -$(R^{13}O)_m$-$CH(R^{15})_2$ wherein said alkyl, alkenyl and alkynyl radicals are optionally substituted with one or more groups independently selected from A1, said aryl radical is optionally substituted with one or more groups independently selected from A3, and said heterocycle is optionally substituted with one or more groups independently selected from A4;

m is an integer ranging from 1-1000;

R^{13} is -C_2-C_{20} alkylene, -C_2-C_{20} alkenylene, or -C_2-C_{20} alkynylene, each of which is optionally substituted with one or more groups independently selected from A1;

R^{14} is -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, or -C_2-C_{20} alkynyl wherein said alkyl, alkenyl and alkynyl radicals are optionally substituted with one or more groups independently selected from A1;

each occurrence of **R^{15}** is independently -H, -COOH, –$(CH_2)_n$-$N(R^{16})_2$, –$(CH_2)_n$-SO_3H, –$(CH_2)_n$-SO_3-C_1-C_{20} alkyl, –$(CH_2)_n$-SO_3-C_2-C_{20} alkenyl, or –$(CH_2)_n$-SO_3-C_2-C_{20} alkynyl wherein said alkyl, alkenyl and alkynyl radicals are optionally substituted with one or more groups independently selected from A1;

each occurrence of **R^{16}** is independently -H, -C_1-C_{20} alkyl, -C_2-C_{20} alkenyl, -C_2-C_{20} alkynyl or –$(CH_2)_n$-COOH wherein said alkyl, alkenyl and alkynyl radicals are optionally substituted with one or more groups independently selected from A1;

n is an integer ranging from 0 to 6; and A1, A2, A3, and A4 are as defined herein; or a pharmaceutically acceptable salt or solvate form thereof.

[0224] In certain of these embodiments, **R^{10}** is phenyl optionally substituted with one or more groups independently selected from A3.

[0225] Auristatins of the formula **D_F** include those wherein the groups of R^2, R^3, R^4, R^5, R^6, R^7, R^8, and R^9 are unsubstituted and the groups of R^{10} and R^{11} are as described herein.

[0226] Auristatins of the formula $\mathbf{D_F}$ include those wherein said alkyl, alkenyl, alkynyl, alkylene, alkenylene, alkynyklene, aryl, carbocyle, and heterocycle radicals are unsubstituted.

[0227] Auristatins of the formula $\mathbf{D_F}$ include those wherein

$\mathbf{R^2}$ is C_1-C_3 alkyl; $\mathbf{R^3}$ is H or C_1-C_3 alkyl; $\mathbf{R^4}$ is C_1-C_5 alkyl; $\mathbf{R^5}$ is H; $\mathbf{R^6}$ is C_1-C_3 alkyl; $\mathbf{R^7}$ is C_1-C_5 alkyl; $\mathbf{R^8}$ is C_1-C_3 alkoxy; $\mathbf{R^9}$ is hydrogen or C_1-C_8 alkyl; $\mathbf{R^{10}}$ is phenyl optionally substituted with one or more groups independently selected from A3;**Z** is O, S, or NH; and R^{11} and A3 is as defined herein; or a pharmaceutically acceptable salt or solvate form thereof.

[0228] Auristatins of the formula $\mathbf{D_F}$ include those wherein

$\mathbf{R^2}$ is methyl; $\mathbf{R^3}$ is H or C_1-C_3 alkyl; $\mathbf{R^4}$ is C_1-C_5 alkyl; $\mathbf{R^5}$ is H; $\mathbf{R^6}$ is methyl; $\mathbf{R^7}$ is isopropyl or sec-butyl; $\mathbf{R^8}$ is methoxy; $\mathbf{R^9}$ is hydrogen or C_1-C_8 alkyl; $\mathbf{R^{10}}$ is phenyl optionally substituted with one or more groups independently selected from A3;**Z** is O, S, or NH; and R^{11} and A3 is as defined herein; or a pharmaceutically acceptable salt or solvate form thereof.

[0229] Auristatins of the formula $\mathbf{D_F}$ include those wherein

$\mathbf{R^2}$ is methyl; $\mathbf{R^3}$ is H or C_1-C_3 alkyl; $\mathbf{R^4}$ is C_1-C_5 alkyl; $\mathbf{R^5}$ is H; $\mathbf{R^6}$ is methyl; $\mathbf{R^7}$ is isopropyl or sec-butyl; $\mathbf{R^8}$ is methoxy; $\mathbf{R^9}$ is hydrogen or C_1-C_8 alkyl; $\mathbf{R^{10}}$ is phenyl; and Z is O or NH and R^{11} is as defined herein, preferably hydrogen; or a pharmaceutically acceptable salt or solvate form thereof.

[0230] Auristatins of the formula $\mathbf{D_F}$ include those wherein

$\mathbf{R^2}$ is C_1-C_3 alkyl; $\mathbf{R^3}$ is H or C_1-C_3 alkyl; $\mathbf{R^4}$ is C_1-C_5 alkyl; $\mathbf{R^5}$ is H; $\mathbf{R^6}$ is C_1-C_3 alkyl; $\mathbf{R^7}$ is C_1-C_5 alkyl; $\mathbf{R^8}$ is C_1-C_3 alkoxy; $\mathbf{R^9}$ is hydrogen or C_1-C_8 alkyl; $\mathbf{R^{10}}$ is phenyl; and **Z** is O or NH and R^{11} is as defined herein, preferably hydrogen; or a pharmaceutically acceptable salt or solvate form thereof.

[0231] Auristatins of the formula $\mathbf{D_E}$ or $\mathbf{D_F}$ include those wherein R^3, R^4 and R^7 are independently isopropyl or sec-butyl and R^5 is -H. In an exemplary embodiment, R^3 and R^4 are each isopropyl, R^5 is H, and R^7 is sec-butyl. The remainder of the substituents are as defined herein.

[0232] Auristatins of the formula $\mathbf{D_E}$ or $\mathbf{D_F}$ include those wherein R^2 and R^6 are each methyl, and R^9 is H. The remainder of the substituents are as defined herein.

[0233] Auristatins of the formula $\mathbf{D_E}$ or $\mathbf{D_F}$ include those wherein each occurrence of R^8 is $-OCH_3$. The remainder of the substituents are as defined herein.

[0234] Auristatins of the formula $\mathbf{D_E}$ or $\mathbf{D_F}$ include those wherein R^3 and R^4 are each isopropyl, R^2 and R^6 are each methyl, R^5 is H, R^7 is sec-butyl, each occurrence of R^8 is $-OCH_3$, and R^9 is H. The remainder of the substituents are as defined herein.

[0235] Auristatins of the formula $\mathbf{D_F}$ include those wherein Z is -O- or -NH-. The remainder of the substituents are as defined herein.

[0236] Auristatins of the formula $\mathbf{D_F}$ include those wherein R^{10} is aryl. The remainder of the substituents are as defined herein.

[0237] Auristatins of the formula $\mathbf{D_F}$ include those wherei, R^{10} is -phenyl. The remainder of the substituents are as defined herein.

[0238] Auristatins of the formula $\mathbf{D_F}$ include those wherein Z is -O-, and R^{11} is H, methyl or t-butyl. The remainder of the substituents are as defined herein.

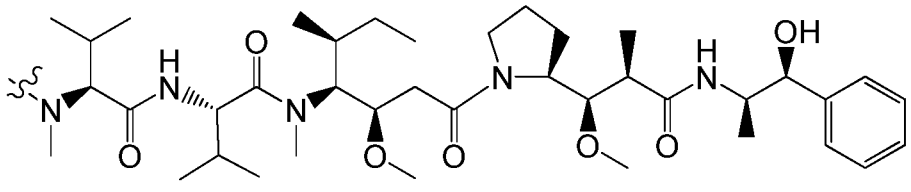
[0239] Auristatins of the formula $\mathbf{D_F}$ include those wherein, when Z is -NH, R^{11} is $-CH(R^{15})_2$, wherein R^{15} is $-(CH_2)_n-N(R^{16})_2$, and R^{16} is $-C_1-C_8$ alkyl or $-(CH_2)_n-COOH$. The remainder of the substituents are as defined herein.

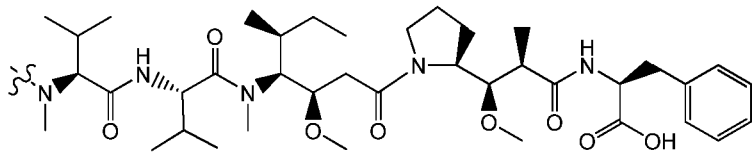
[0240] Auristatins of the formula $\mathbf{D_F}$ include those wherein when Z is -NH, R^{11} is $-CH(R^{15})_2$, wherein R^{15} is $-(CH_2)_n-SO_3H$. The remainder of the substituents are as defined herein.

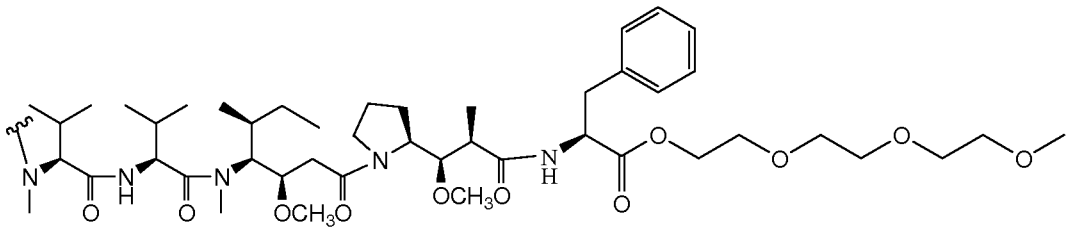
[0241] In preferred embodiments, when D is an auristatin of formula $\mathbf{D_E}$, w is an integer ranging from 1 to 12, preferably 2 to 12, y is 1 or 2, and a is preferably 1.

[0242] In some embodiments, wheren D is is an auristatin of formula $\mathbf{D_F}$, a is 1 and w and y are 0.

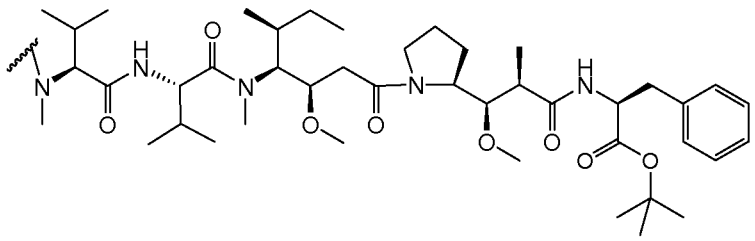
[0243] Illustrative Drug units (-D) include the drug units having the following structures:



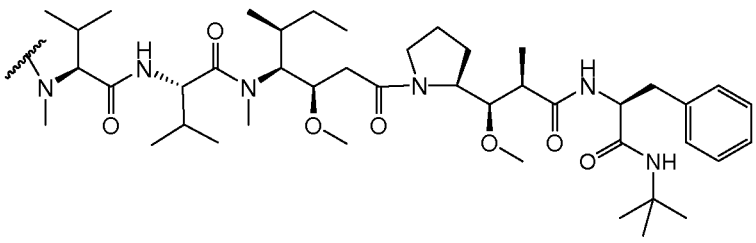




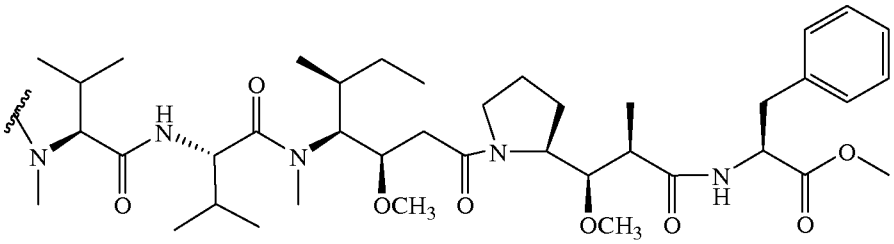
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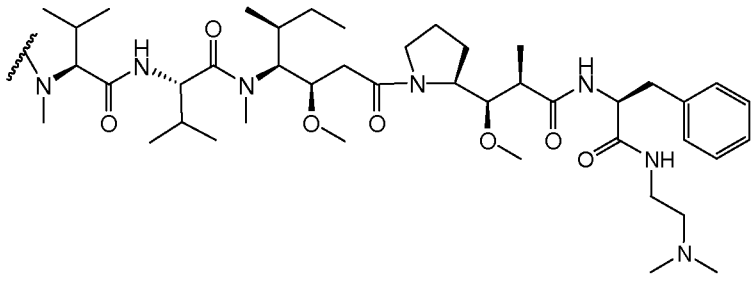
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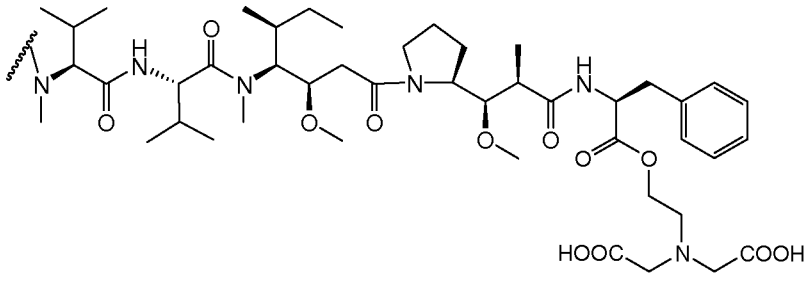
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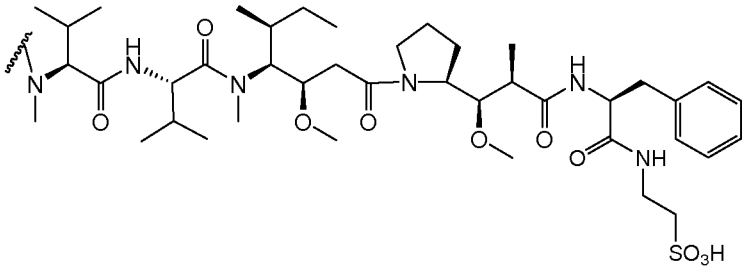
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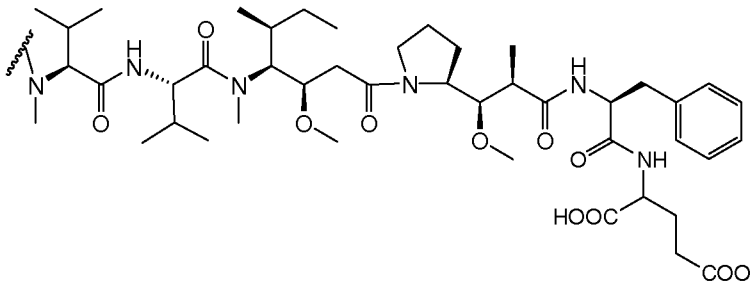
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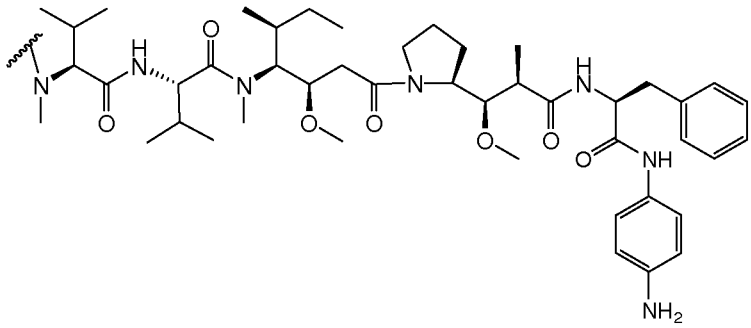
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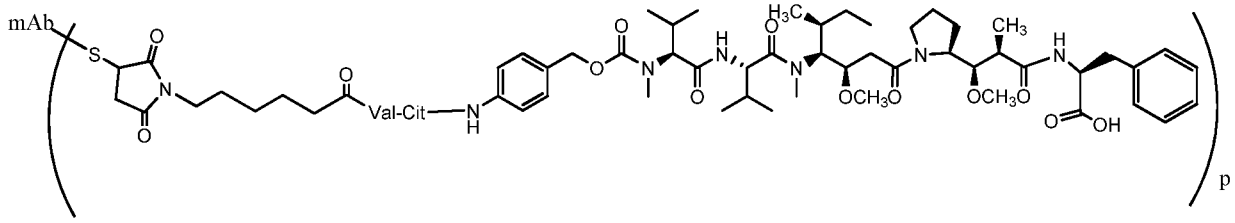


or pharmaceutically acceptable salts or solvates thereof.

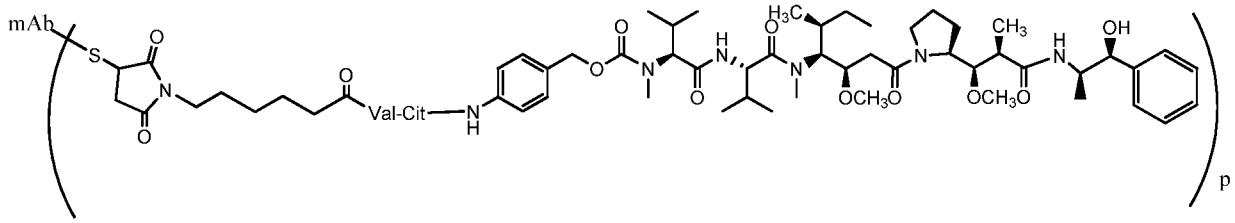
[0244] In one aspect, hydrophilic groups, such as but not limited to triethylene glycol esters (TEG) can be attached to the Drug Unit at R^{11}. Without being bound by theory, the hydrophilic groups assist in the internalization and non-agglomeration of the Drug Unit.

[0245] In some embodiments, the Drug unit is not TZT-1027. In some embodiments, the Drug unit is not auristatin E, dolastatin 10, or auristatin PE.

[0246] Exemplary antibody-drug conjugate compounds have the following structures wherein "mAb-s-" represents the anti-CD30 antibody:

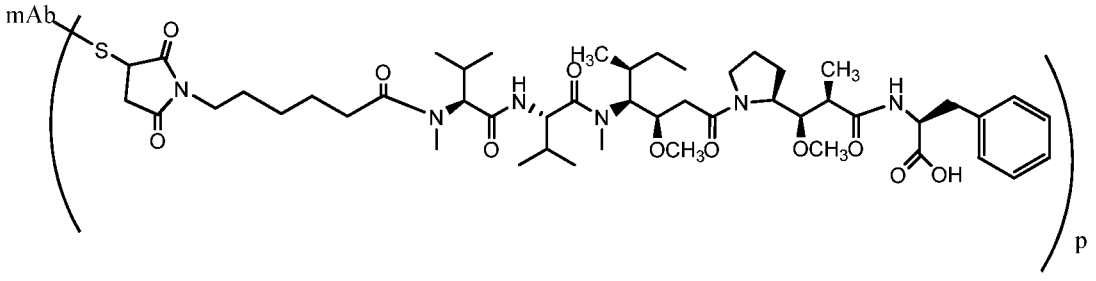


L-MC-vc-PAB-MMAF



L-MC-vc-PAB-MMAE

or



L-MC-MMAF

wherein Val is valine, and Cit is citrulline.

ANTIBODY UNIT

[0247] The antibody unit (L) has at least one functional group that can form a bond with a functional group of a Linker unit or a Drug unit. Useful functional groups that can be present on an antibody unit, either naturally, via chemical manipulation or via engineering, include, but are not limited to, sulfhydryl (-SH), amino, hydroxyl, carboxy, the anomeric hydroxyl group of a carbohydrate, and carboxyl. In some embodiments, an antibody unit functional group is a sulfhydryl group. The sulfhydryl group is typically a solvent accessible sulfhydryl group, such as a solvent accessible sulfhydryl group on a cysteine residue. Sulfhydryl groups can be generated by reduction of an intramolecular or intermolecular disulfide bond of an antibody. Sulfhydryl groups also can be generated by reaction of an amino group of a lysine moiety of an antibody using 2-iminothiolane (Traut's reagent) or another sulfhydryl generating reagent.

[0248] In some embodiments, one or more sulfhydryl groups are engineered into an antibody unit, such as by amino acid substitution. For example, a sulfhydryl group can be introduced into an antibody unit. In some embodiments, a sulfhydryl group is introduced by an amino acid substitution of serine or threonine to a cysteine residue, and/or by addition of a cysteine residue into an antibody unit (an engineered cysteine residue). In some embodiments, the cysteine residue is an internal cysteine residue, *i.e.*, not located at the N-terminus or C-terminus of the antibody moiety.

[0249] To control the number of Drug or Linker unit-Drug units attached to an antibody unit, one or more cysteine residues can be eliminated by amino acid substitution. For example, the number of solvent accessible cysteine residues in an immunoglobulin hinge region can be reduced by amino acid substitution of cysteine to serine residues.

[0250] In some embodiments, an antibody unit contains 1, 2, 3, 4, 5, 6 7 or 8 solvent-accessible cysteine residues. In some embodiments, an antibody unit contains 2 or 4 solvent-accessible cysteine residues.

[0251] The present invention also provides kits for the treatment of HL. The kit can comprise (a) a container containing an antibody-drug conjugate and (b) one or more additional containers containing the chemotherapeutic drugs. Such kits can further include, if desired, one or more of various conventional pharmaceutical kit components, such as, for example, containers with one or more pharmaceutically

acceptable carriers, additional containers, etc., as will be readily apparent to those skilled in the art. Printed instructions, either as inserts or as labels, indicating quantities of the components to be administered, guidelines for administration, and/or guidelines for mixing the components, can also be included in the kit.

[0252] The present invention is not to be limited in scope by the specific embodiments described herein. Various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

[0253] All publications and patent documents cited above are hereby incorporated by reference in their entirety for all purposes to the same extent as if each were so individually denoted.

[0254] The invention is further described in the following examples, which are in not intended to limit the scope of the invention.

EXAMPLES

Example 1: Combination of the antibody-drug conjugate cAC10-vcMMAE (cAC10-MC-vc-PAB-MMAE) with chemotherapeutic regimens for the treatment of HL

[0255] The effects of combining cAC10-vcMMAE treatment with gemcitabine or ABVD or other chemotherapeutic regimens were studied in a L540cy tumor model (Francisco et al., Blood 2003;102:1458-146). To determine the maximum tolerated dose (MTD) of ABVD and gemcitabine, body weights of SCID mice treated with increasing amounts of drugs were assessed daily. The criteria defining the MTD were $\geq$ 20% decrease in body weights or other signs of morbidity during the entire treatment followed by a 2 week recovery period. Tumor quadrupling or triplicating times were chosen as time to endpoint (TTE), which were determined by using the non-liner regression analysis for exponential growth of each individual tumor growth data set from each experimental animal. The tumor quadrupling time was calculated based on the tumor volume at the beginning of treatment. Animals that did not reach the endpoint were assigned a TTE value equal to the last day of the study. % TGD (tumor growth delay) reflects the delay in reaching TTE relative to control treated tumors, which was determined by using the formula: %TGD= [(T-C)/C]x100, where T and C are the median times in days for treated and control groups, to reach TTE, using the start of treatment as day 1. Statistical analysis and graphic presentations were conducted using Prism (GraphPad) software for Windows 3.03 software. Median tumor growth curves show group median tumor volumes as a function of time. The Logrank test was used to analyze the significance of the differences between TTE of treated and control tumor groups, with differences deemed significant (*) at $0.01 \leq P \leq 0.05$, and highly significant (**) at $P < 0.01$. A 1 mg/kg, q4dx3 treatment schedule for cAC10-vcMMAE was selected based on previous reports demonstrating maximal therapeutics effects at a q4d x4 schedule.

[0256] Administration of ABVD or cAC10-vcMMAE alone to L540cy tumor bearing mice induced tumor regressions and significant tumor growth delays compared to control treatment (Figure 1A). However, tumors eventually progressed. There were 4/9 durable tumor regressions in the animals treated with cAC10-vcMMAE monotherapy and 0/9 durable responses in the animals treated with ABVD monotherapy. In contrast, combination of cAC10-vcMMAE with ABVD resulted in durable tumor regressions in all experimental animals (9/9 durable responses) (Fig.

1A) and a statistically significant increase in tumor growth delay relative to each treatment arm alone. Similarly, when treatment was initiated at 300 mm^3 tumor volume, there was a significant increase in TGD and 50% durable responses (5/10 animals) in the animals treated with combination therapy (Figure 1B), whereas there were 2/10 durable responses in animals treated with cAC10-vcMMAE monotherapy and no durable responsese in animals treated with ABVD monotherapy. It should be noted that the dosages for the ABVD regimen were reduced by 25% in the high-bar model as compared to the low-bar model. The delay in tumor growth induced by the combination treatment was highly significant relative to each individual treatment arm alone (Table 1). No significant differences in body weight loss or morbidity were noted in the combination group, suggesting comparable tolerability (data not shown).

Table 1

Treatment	Median TGD (Figure 1A) (days to quadruple)	Median TGD (Figure 1B) (days to triple)	Combo vs single agent (P-vlaue) (A) (B)
cAC10-vcMMAE	63	41	p=0.0101 p=0.05
ABVD	38.4	22.5	p<0.0001 p=0.001
ABVD + cAC10-vcMMAE	>80.5*	61.5	

*The experiment was terminated. At this point, there were durable tumor regressions in all animals.

[0257] Next, the effect of combining cAC10-vcMMAE with gemcitabine was studied. For this purpose, mice were implanted with L540cy tumors and treated with cAC10-vcMMAE and gemcitabine, either alone or combined. While single arm treatment led to significant delays in tumor growth and one durable response, the combination of cAC10-vcMMAE with gemcitabine enhanced the anti-tumor response and durable responses were found in all animals (5/5, Fig. 2A). Improved efficacy in the combination treatment group was also noted when drug administration occurred when tumors reached a substantially larger size (300 mm^3, Fig. 2B). Immunohistochemical analysis of tumors did not reveal significant changes in CD30

expression levels in tumors treated with either ABVD or gemcitabine, ruling out drug interference with target gene expression (data not shown). Similar to the ABVD experiment, combination treatment with gemcitabine resulted in a significant delay in tumor growth, which was more than additive (Table 2) and lead to durable responses. No significant differences in body weight loss or morbidity were noted in the combination group, suggesting comparable tolerability (data not shown).

Table 2

Treatment	Median TGD (Figure 2A) (days to quadruple)	Median TGD (Figure 2B) (days to triplicate)	Combo vs single agent (P-vlaue) (A) (B)
cAC10-vcMMAE	34	39.5	p=0.0088 p=0.0375
Gemcitabine	5	15.5	p<0.0024 p=0.0154
Gemcitabine + cAC10-vcMMAE	>75	>75	

[0258] In a second study illustrated by Figure 7 and Table 3 below, 7/8 durable responses were seen with combination treatment with cAC10-vcMMAE and gemcitabine, whereas 1/7 durable responses were seen with single arm treatment with cAC10-vcMMAE and 1/6 durable responses were seen with single arm treatment with gemcitabine.

Table 3

Treatment	Median TGD (Figure 7) (days to quadruple)	Combo vs single agent (P-vlaue) (A)
cAC10-vcMMAE	78.01	p=0.0037
Gemcitabine	42	P=0.0021
Gemcitabine +	>100*	

cAC10-vcMMAE		

*The experiment was terminated. At this point, there were durable tumor regressions in 7/8 animals.

[0259] The effect of combining cAC10-vcMMAE with GVD was studied. For this purpose, mice were implanted with L540cy tumors and treated with cAC10-vcMMAE and GVD, either alone or combined. Treatment was initiated at 100 mm^3 tumor volume for two separate experiments that were conducted (3A and 3B). In the first experiment, 6/9 durable responses were seen with the combination therapy whereas 4/9 durable responses were seen with cAC10-vcMMAE treatment alone and 0/9 durable responses with single arm treatment with GVD. In the second experiment, 7/7 durable responses were seen with the combination therapy whereas 0/7 durable responses were seen with cAC10-vcMMAE treatment alone and 0/7 durable responses with single arm treatment with GVD.

Table 4

Treatment	Median TGD (Figure 3A) (days to quadruple)	Median TGD (Figure 3B) (days to quadruple)	Combo vs single agent (P-vlaue) (A) (B)
cAC10-vcMMAE	84.6	54.7	p=0.5089 p=0.0004
GVD	33.2	34.4	p<0.0001 p=0.0005
GVD + cAC10-vcMMAE	>77	>107	

[0260] The effect of combining cAC10-vcMMAE with vinorelbine was studied. For this purpose, mice were implanted with L540cy tumors and treated with cAC10-vcMMAE and vinorelbine, either alone or combined. Treatment was initiated at 100 mm^3 tumor volume for two separate experiments that were conducted (4A and 4B). In the first experiment, 1/6 durable responses were seen with the combination therapy whereas 1/6 durable responses were seen with cAC10-vcMMAE treatment

alone and 0/6 durable responses with single arm treatment with vinorelbine. In the second experiment, 6/10 durable responses were seen with the combination therapy whereas 4/10 durable responses were seen with cAC10-vcMMAE treatment alone and 0/9 durable responses with single arm treatment with vinorelbine

Table 5

Treatment	Median TGD (Figure 4A) (days to quadruple)	Median TGD (Figure 4B) (days to quadruple)	Combo vs single agent (P-vlaue) (A) (B)
cAC10-vcMMAE	64	78.8	p=0.4500 p=0.3769
Vinorelbine	28	34.4	p<0.0004 p=0.0001
Vinorelbine + cAC10-vcMMAE	71	>78	

[0261] The effect of combining cAC10-vcMMAE with doxorubicin was studied. For this purpose, mice were implanted with L540cy tumors and treated with cAC10-vcMMAE and doxorubicin, either alone or combined. Treatment was initiated at 100 mm^3 tumor volume for two separate experiments that were conducted (5A and 5B). In the first experiment, 0/4 durable responses were seen with the combination therapy whereas 1/6 durable responses were seen with cAC10-vcMMAE treatment alone and 0/3 durable responses with single arm treatment with doxorubicin. In the second experiment, 7/10 durable responses were seen with the combination therapy whereas 4/10 durable responses were seen with cAC10-vcMMAE treatment alone and 0/9 durable responses with single arm treatment with doxorubicin

Table 6

Treatment	Median TGD (Figure 5A) (days to quadruple)	Median TGD (Figure 5B) (days to quadruple)	Combo vs single agent (P-vlaue) (A) (B)

cAC10-vcMMAE	64	78.8	p=0.0140 p=0.0762
Doxorubicin	32	27.2	p<0.0067 p=0.0001
Doxorubicin + cAC10-vcMMAE	52	> 78	

[0262] The effect of combining cAC10-vcMMAE with vinblastine was studied. For this purpose, mice were implanted with L540cy tumors and treated with cAC10-vcMMAE and vinblastine, either alone or combined. Treatment was initiated at 300 mm^3 tumor volume (6). 0/10 durable responses were seen with the combination therapy whereas 1/8 durable responses were seen with cAC10-vcMMAE treatment alone and 0/10 durable responses with single arm treatment with vinblastine.

Table 7

Treatment	Median TGD (Figure 6) (days to triple)	Combo vs single agent (P-vlaue)
cAC10-vcMMAE	45.9	p=0.5970
Vinblastine	30.93	p<0.0001
Vinblastine + cAC10-vcMMAE	55.7	

Example 2: Combination of the antibody-drug conjugate cAC10-mcMMAF with gemcitabine for the treatment of HL

[0263] The effects of combining cAC10-mcMMAF treatment with gemcitabine was studied in the same manner as the experiments with cAC10-vcMMAE. A 1 mg/kg, q4dx3 treatment schedule for cAC10-mcMMAF was selected.

At day 51 of the experiment, 9/10 durable responses were seen with the combination therapy whereas 0/10 durable responses were seen with cAC10-mcMMAF treatment alone and 0/10 durable responses with single arm treatment with gemcitabine. The experiment is in its 51rst day and is not yet complete.

Claims

1. The use of an antibody-drug conjugate compound comprising an anti-CD30 antibody conjugated to an auristatin compound in the manufacture of a medicament for use in combination therapy with a chemotherapeutic regimen comprising doxorubicin, bleomycin, vinblastine, and dacarbazine for the treatment of Hodgkin lymphoma in a subject.

2. The use of claim 1 wherein said antibody-drug conjugate compound is for delivery over a treatment cycle wherein the total dose over the treatment cycle is from about 0.1 mg/kg to about 3.8 mg/kg of the subject's body weight.

3. The use of claim 1 wherein said antibody-drug conjugate compound is for delivery over a treatment cycle wherein the total dose over the treatment cycle is from 0.1 mg/kg to 2.7 mg/kg of the subject's body weight.

4. The use of any one of claims 2 to 3 wherein the antibody-drug conjugate compound is delivered as a split dose over the treatment cycle.

5. The use of any one of claims 2 to 3 wherein the antibody-drug conjugate compound is delivered as a single dose over the treatment cycle.

6. The use of any one of claims 2 to 4 wherein the treatment cycle is three or four weeks.

7. The use of claim 6 wherein said chemotherapeutic regimen and said antibody-drug conjugate compound are administered on days 1 and 15 of a four week treatment cycle.

8. The use of claim 1 wherein said antibody-drug conjugate compound is administered in a dose range of 0.2 to 1.2 mg/kg of the subject's body weight per dose.

9. The use of claim 1 wherein said antibody-drug conjugate compound is administered in a dose range of 1 to 1.5 mg/kg of the subject's body weight per dose.

10. The use of any one of claims 1 to 9 wherein said subject is treated with about 25 mg/m^2 doxorubicin, about 10 U/m^2bleomycin, about 6 mg/m^2 vinblastine, and about 375 mg/m^2dacarbazine on days 1 and 15 of a four week treatment cycle.

11. The use of any one of claims 1 to 6 and 8 to 9 wherein the antibody-drug conjugate compound and chemotherapeutic regimen comprising doxorubicin, bleomycin, vinblastine and dacarbazine are administered during a treatment cycle of three or four weeks and no additional anti-cancer agents are administered during the treatment cycle.

12. The use of claim 1, consisting essentially of administering to a subject in need thereof a chemotherapeutic regimen comprising doxorubicin, bleomycin, vinblastine and dacarbazine and an antibody-drug conjugate compound, wherein said antibody-drug conjugate compound is an anti-CD30 antibody conjugated to an auristatin compound and administration of said antibody-drug conjugate compound and the chemotherapeutic regimen provides a synergistic effect in the treatment of Hodgkin lymphoma in the patient.

13. The use of any one of claims 1 to 12 wherein said subject has relapsed or refractory Hodgkin lymphoma and has not previously been treated with said chemotherapeutic regimen.

14. The use of any one of claims 1 to 13 wherein the antibody-drug conjugate compound is administered for two or more treatment cycles.

15. The use of an antibody-drug conjugate compound comprising an anti-CD30 antibody conjugated to an auristatin compound in the manufacture of a medicament for use in combination therapy with gemcitabine for the treatment of Hodgkin lymphoma in a subject.

16. The use of claim 15 wherein said antibody-drug conjugate compound is delivered over a treatment cycle wherein the total dose over the treatment cycle is from 0.1 mg/kg to 3.2 mg/kg of the subject's body weight.

17. The use of claim 15 wherein said antibody-drug conjugate compound is delivered over a treatment cycle wherein the total dose over the treatment cycle is from about 0.6 mg/kg to about 3.2 mg/kg of the subject's body weight.

18. The use of any one of claims 16 to 17 wherein the antibody-drug conjugate compound is delivered as a split dose over the treatment cycle.

19. The use of any one of claims 16 to 17 wherein the antibody-drug conjugate compound is delivered as a single dose over the treatment cycle.

20. The use of any one of claims 16 to 19 wherein the treatment cycle is three weeks.

21. The use of any one of claims 16 to 19 wherein the treatment cycle is four weeks.

22. The use of claim 15 wherein the antibody-drug conjugate compound is administered in a dose range of 0.4 mg/kg to 1 mg/kg of the subject's body weight per dose.

23. The use of any one of claims 15 to 22 wherein gemcitabine is administered in a dose range of 500 mg/m^2 to 1500 mg/m^2 per dose.

24. The use of any one of claims 15 to 19 and 22 to 23 wherein the antibody-drug conjugate compound and gemcitabine are administered during a treatment cycle of three or four weeks and no additional anti-cancer agents are administered during the treatment cycle.

25. The use of claim 15, consisting essentially of administering to a subject in need thereof gemcitabine and an antibody-drug conjugate compound, wherein said antibody-drug conjugate compound is an anti-CD30 antibody conjugated to an auristatin compound and administration of said antibody-drug conjugate compound and gemcitabine provides a synergistic effect in the treatment of Hodgkin lymphoma in the patient.

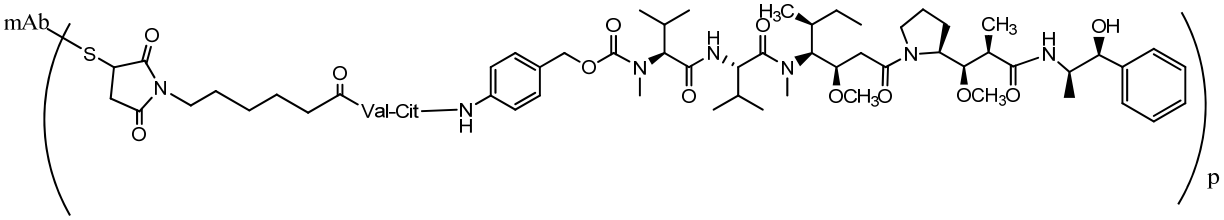
26. The use of any one of claims 15 to 25 wherein said subject is suffering from advanced stage Hodgkin lymphoma.

27. The use of any one of claims 15 to 25 wherein said subject has relapsed or refractory Hodgkin lymphoma.

28. The use of any one of claims 15 to 19 and 22 to 27 wherein the antibody-drug conjugate compound is administered for two or more treatment cycles of three or four weeks.

29. The use of any one of claims 1 to 28 wherein the auristatin compound is conjugated to the anti-CD30 antibody via a linker that is cleavable under intracellular conditions, such that cleavage of the linker releases the auristatin compound from the antibody in the intracellular environment.

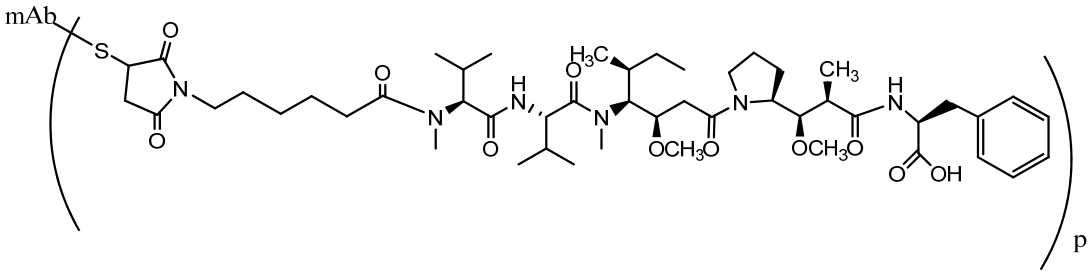
30. The use of any one of claims 1 to 28 wherein the antibody-drug conjugate compound has the following structure:



whereinmAb-s- represents the anti-CD30 antibody and p ranges from 1 to about 10.

31. The use of any one of claims 1 to 28 wherein the auristatin compound is conjugated to the antiCD30 antibody via a linker that is not cleavable under intracellular conditions and the auristatin compound is released by antibody degradation.

32. The use of any one of claims 1 to 28 wherein the antibody-drug conjugate compound has the following structure:



whereinmAb-s- represents the anti-CD30 antibody and p ranges from 1 to about 10.

33. The use of claim 30 or 32 wherein there is an average of 4 auristatin drugs per antibody.

34. The use of any one of claims 1 to 33 wherein said anti-CD30 antibody is a chimeric AC10 antibody or competes for binding with a chimeric AC10 antibody.

35. The use of claim 34 wherein the anti-CD30 antibody is a chimeric AC10 antibody.

ST25

SEQUENCE LISTING

<110> Seattle Genetics, Inc.
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Sievers, Eric
Gerber, Hans-Peter

<120> Combination Therapy With Antibody-Drug Conjugates

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<151> 2007-10-12

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            20                  25                  30

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    50                  55                  60

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65                  70                  75                  80

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65                  70                  75                  80

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65                  70                  75                  80

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Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
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