

[0012] Pertuzumab has been evaluated in Phase II studies in combination with trastuzumab in patients with HER2-positive metastatic breast cancer who have previously received trastuzumab for metastatic disease. One study, conducted by the National cancer Institute (NCI), enrolled 11 patients with previously treated HER2-positive metastatic breast cancer. Two out of the 11 patients exhibited a partial response (PR) (Baselga et al., J Clin Oncol 2007 ASCO Annual Meeting Proceedings; 25:18 S (June 20 Supplement): 1004. The results of a Phase II neoadjuvant study evaluating the effect of a novel combination regimen of pertuzumab and trastuzumab plus chemotherapy (Docetaxel) in women with early-stage HER2-positive breast cancer, presented at the CTRC-AACR San Antonio Breast Cancer Symposium (SABCS), Dec. 8-12, 2010, showed that the two HER2 antibodies plus Docetaxel given in the neoadjuvant setting prior to surgery significantly improved the rate of complete tumor disappearance (pathological complete response rate, pCR, of 45.8 percent) in the breast by more than half compared to trastuzumab plus Docetaxel (pCR of 29.0 percent), $p=0.014$.

[0013] Pertuzumab, marketed under the tradename PERJETA®, was approved in 2012 for the treatment of patients with advanced or late-stage (metastatic) HER2-positive breast cancer. HER2-positive breast cancers have increased amounts of the HER2 protein that contributes to cancer cell growth and survival.

[0014] On September 30, 2013, the U.S. Food and Drug Administration granted accelerated approval to PERJETA® (pertuzumab) as part of a complete treatment regimen for patients with early stage breast cancer (EBC) before surgery (neoadjuvant setting). PERJETA® is the first FDA-approved drug for the neoadjuvant treatment of breast cancer.

[0015] There is a need in the art for additional safe and effective agents that target HER2 for treatment of HER2-associated conditions, such as breast cancer, for use in monotherapy and combination therapy. The invention fulfills that need and provides other benefits.

SUMMARY

[0016] The disclosure provides anti-HER2 antibodies and immunoconjugates and methods of using the same.

[0017] In one aspect, an isolated antibody that binds to HER2 is provided, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:15; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:16; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:17; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:12; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:13; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:14. In another aspect, an isolated antibody that binds to HER2 is provided, wherein the antibody comprises a heavy chain variable region comprising the sequence of SEQ ID NO: 11 and a light chain variable region comprising the sequence of SEQ ID NO: 10. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a

additional therapeutic agent is (i) an antibody or immunoconjugate that binds to domain II of HER2, and/or (ii) an antibody or immunoconjugate that binds to domain IV or HER2. In some embodiments, the additional therapeutic agent is (i) an antibody or immunoconjugate that binds to epitope 2C4, and/or (ii) an antibody or immunoconjugate that binds to epitope 4D5. In some embodiments, the additional therapeutic agent is selected from trastuzumab, trastuzumab-MCC-DM1 (T-DM1), and pertuzumab. In some embodiments, the pharmaceutical formulation further comprises (1) trastuzumab or T-DM1, and (2) pertuzumab.

[0033] In one aspect, use of the immunoconjugate described herein in the manufacture of a medicament for treating an individual having a HER2-positive cancer is provided. In another aspect, use of the pharmaceutical formulation described herein in the manufacture of a medicament for treating an individual having a HER2-positive cancer is provided. In some embodiments, methods of treating an individual having a HER2-positive cancer are provided. In some embodiments, a method comprises administering to the individual an effective amount of an immunoconjugate described herein, or a pharmaceutical composition described herein. In some embodiments, the HER2-positive cancer is breast cancer or gastric cancer. In some embodiments, the HER2-positive breast cancer is early-stage breast cancer. In some embodiments, the HER2-positive breast cancer is metastatic breast cancer. In some embodiments, the HER2-positive cancer is recurrent cancer. In some embodiments, the recurrent cancer is locally recurrent cancer. In some embodiments, the HER2-positive cancer is advanced cancer. In some embodiments, the HER2-positive cancer is non-resectable. In some embodiments, the method further comprises administering an additional therapeutic agent to the individual.

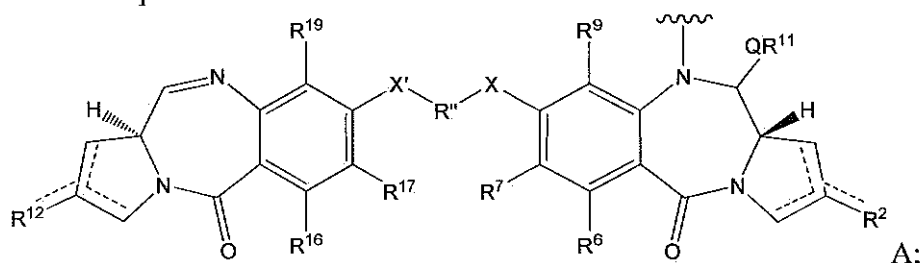
[0034] In some embodiments, a method of treating an individual having a HER2-positive cancer comprises administering to the individual an effective amount of an immunoconjugate described herein and at least one additional therapeutic agent to the individual. In some embodiments, the additional therapeutic agent is an antibody or immunoconjugate that binds to HER2. In some embodiments, the additional therapeutic agent is (i) an antibody or immunoconjugate that binds to domain II of HER2, and/or (ii) an antibody or immunoconjugate that binds to domain IV or HER2. In some embodiments, the additional therapeutic agent is (i) an antibody or immunoconjugate that binds to epitope 2C4, and/or (ii) an antibody or immunoconjugate that binds to epitope 4D5. In some embodiments, the additional therapeutic agent is selected from trastuzumab, trastuzumab-MCC-DM1 (T-DM1), and pertuzumab. In some embodiments, the additional therapeutic agents are (1) trastuzumab or T-DM1, and (2) pertuzumab. In some embodiments, the HER2-positive cancer is breast cancer or gastric cancer. In some embodiments, the HER2-positive breast cancer is early-stage breast cancer. In some embodiments, the HER2-positive breast cancer is metastatic breast cancer. In some embodiments, the HER2-positive cancer is recurrent cancer. In some embodiments, the recurrent cancer is locally recurrent cancer. In some embodiments, the HER2-positive cancer is advanced cancer. In some embodiments, the HER2-positive cancer is non-resectable.

[0035] In one aspect, use of the immunoconjugate described herein in the manufacture of a medicament for treating an individual having a HER2-positive cancer is provided, wherein the immunoconjugate described herein is to be administered to the individual as neoadjuvant treatment, and subsequently as adjuvant treatment after said cancer has been removed by definitive surgery. In another aspect, use of the pharmaceutical formulation described herein in the manufacture of a medicament for treating an individual having a HER2-positive cancer is provided, wherein the pharmaceutical formulation described herein is to be administered to the individual as neoadjuvant treatment, and subsequently as adjuvant treatment after said cancer has been removed by definitive surgery. In some embodiments, a method of treating an individual having a HER2-positive cancer comprises:

WHAT IS CLAIMED IS:

1. An isolated antibody that binds to HER2, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:15; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:16; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:17; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:12; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:13; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:14.
2. An isolated antibody that binds to HER2, wherein the antibody comprises a heavy chain variable region comprising the sequence of SEQ ID NO: 11 and a light chain variable region comprising the sequence of SEQ ID NO: 10.
3. The antibody of any one of the preceding claims, which is a monoclonal antibody.
4. The antibody of any one of the preceding claims, which is a humanized antibody.
5. The antibody of any one of claims 1 to 3, which is a chimeric antibody.
6. The antibody of any one of the preceding claims, which is an antibody fragment that binds HER2.
7. The antibody of any one of the preceding claims, wherein said HER2 is human HER2 comprising amino acids 23 to 1255 of SEQ ID NO: 1.
8. The antibody of any one of the preceding claims, wherein the antibody binds to extracellular domain I of HER2.
9. The antibody of claim 7, wherein the extracellular domain I of HER2 has the sequence of SEQ ID NO: 35.
10. The antibody of any one of the preceding claims, which is an IgG1, IgG2a or IgG2b antibody.
11. The antibody of any one of claims 1 to 10, wherein the antibody comprises at least one mutation in the heavy chain constant region selected from A118C and S400C.
12. The antibody of any one of claims 1 to 10, wherein the antibody comprises at least one mutation in the light chain constant region selected from K149C and V205C.
13. The antibody of any one of claims 1 to 10, wherein the antibody comprises:
 - a) a heavy chain comprising the sequence of SEQ ID NO: 19 and a light chain comprising the sequence of SEQ ID NO: 18; or
 - b) a heavy chain comprising the sequence of SEQ ID NO: 19 and a light chain comprising the sequence of SEQ ID NO: 23; or
 - c) a heavy chain comprising the sequence of SEQ ID NO: 24 and a light chain comprising the sequence of SEQ ID NO: 18.
14. The antibody of any one of claims 1 to 10, wherein the antibody comprises the heavy chain constant region of SEQ ID NO: 28.
15. The antibody of any one of claims 1 to 10, wherein the antibody comprises the heavy chain constant region of SEQ ID NO: 26.
16. The antibody of any one of claims 1 to 10, wherein the antibody comprises the light chain constant region of SEQ ID NO: 25.

17. The antibody of any one of claims 1 to 10, wherein the antibody comprises the light chain constant region of SEQ ID NO: 27.
18. An isolated antibody that binds to HER2, wherein the antibody comprises a heavy chain comprising the sequence of SEQ ID NO: 19 and a light chain comprising the sequence of SEQ ID NO: 23.
19. An isolated antibody that binds to HER2, wherein the antibody comprises a heavy chain comprising the sequence of SEQ ID NO: 24 and a light chain comprising the sequence of SEQ ID NO: 18.
20. An isolated antibody that binds to HER2, wherein the antibody comprises a heavy chain comprising the sequence of SEQ ID NO: 19 and a light chain comprising the sequence of SEQ ID NO: 18.
21. An isolated nucleic acid encoding the antibody of any one of the preceding claims.
22. A host cell comprising the nucleic acid of claim 21.
23. A method of producing an antibody comprising culturing the host cell of claim 22 so that the antibody is produced.
24. An immunoconjugate comprising the antibody of any one of claims 1 to 20 and a cytotoxic agent.
25. The immunoconjugate of claim 24 having the formula Ab-(L-D)p, wherein:
 - (a) Ab is the antibody of any one of claim 1 to 20;
 - (b) L is a linker;
 - (c) D is a cytotoxic agent; and
 - (d) p ranges from 1-8.
26. The immunoconjugate of claim 25, wherein p ranges from 1.3-2.
27. The immunoconjugate of claim 25, wherein p is about 2.
28. The immunoconjugate of any one of claims 24 to 27, wherein the cytotoxic agent is selected from an auristatin, a maytansinoid, a calicheamicin, a pyrrolobenzodiazepine, a nemorubicin derivative, and a 1-(chloromethyl)-2,3-dihydro-1H-benzo[e]indole (CBI).
29. The immunoconjugate of any one of claims 24 to 27, wherein the cytotoxic agent is a pyrrolobenzodiazepine of Formula A:



wherein the dotted lines indicate the optional presence of a double bond between C1 and C2 or C2 and C3;

R² is independently selected from H, OH, =O, =CH₂, CN, R, OR, =CH-R^D, =C(R^D)₂, O-SO₂-R, CO₂R and COR, and optionally further selected from halo or dihalo, wherein R^D is independently selected from R, CO₂R, COR, CHO, CO₂H, and halo;

R⁶ and R⁹ are independently selected from H, R, OH, OR, SH, SR, NH₂, NHR, NRR', NO₂, Me₃Sn and halo;

R⁷ is independently selected from H, R, OH, OR, SH, SR, NH₂, NHR, NRR', NO₂, Me₃Sn and halo;

Q is independently selected from O, S and NH, and

R^{11} is either H, or R; or Q is O and R^{11} is SO_3M , where M is a metal cation;

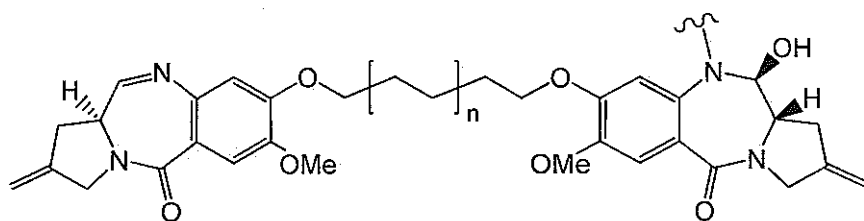
R and R' are each independently selected from optionally substituted C_{1-8} alkyl, C_{3-8} heterocyclyl and C_{5-20} aryl groups, and optionally in relation to the group NRR' , R and R' together with the nitrogen atom to which they are attached form an optionally substituted 4-, 5-, 6- or 7-membered heterocyclic ring;

R^{12} , R^{16} , R^{19} and R^{17} are as defined for R^2 , R^6 , R^9 and R^7 respectively;

R'' is a C_{3-12} alkylene group, which chain may be interrupted by one or more heteroatoms and/or aromatic rings that are optionally substituted; and

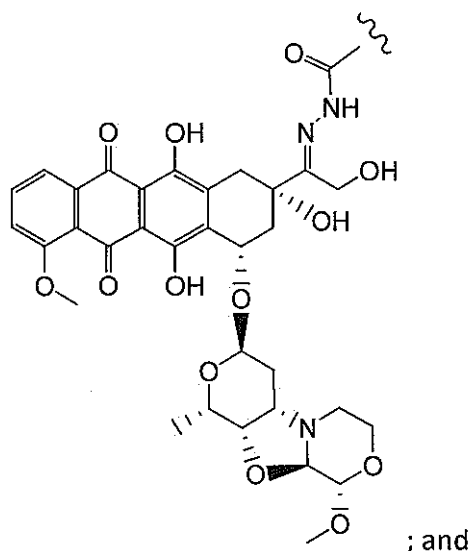
X and X' are independently selected from O, S and N(H).

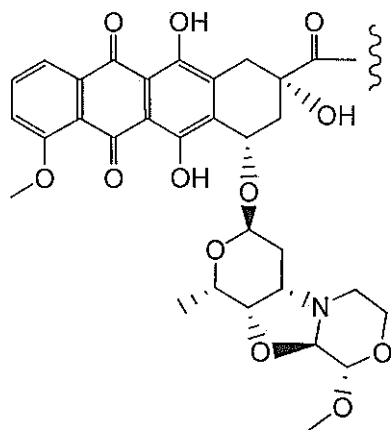
30. The immunoconjugate of claim 29, wherein the cytotoxic agent has the structure:



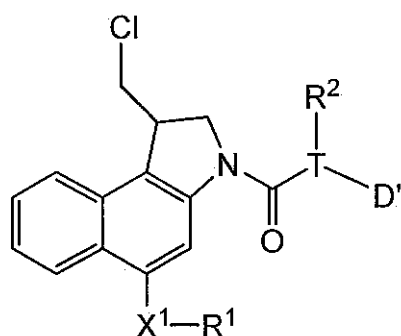
wherein n is 0 or 1.

31. The immunoconjugate of any one of claims 24 to 27, wherein the cytotoxic agent is a nemorubicin derivative.
32. The immunoconjugate of claim 31, wherein the cytotoxic agent has a structure selected from:





33. The immunoconjugate of any one of claims 24 to 27, wherein the cytotoxic agent comprises a 1-(chloromethyl)-2,3-dihydro-1H-benzo[e]indole (CBI).
34. The immunoconjugate of claim 33, wherein the cytotoxic agent has the formula:



where

R^1 is selected from H, $P(O)_3H_2$, $C(O)NR^aR^b$, or a bond to L;

R^2 is selected from H, $P(O)_3H_2$, $C(O)NR^aR^b$, or a bond to L;

R^a and R^b are independently selected from H and C_1-C_6 alkyl optionally substituted with one or more F,

or R^a and R^b form a five or six membered heterocyclyl group;

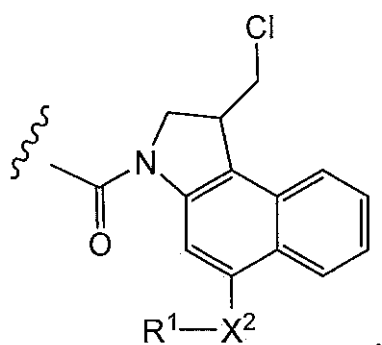
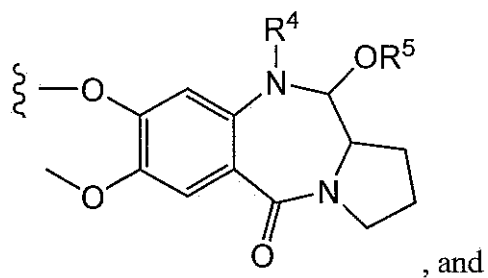
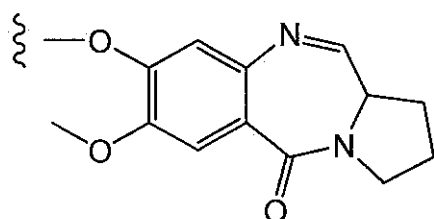
T is a tether group selected from C_3-C_{12} alkylene, Y, $(C_1-C_6 \text{ alkylene})-Y-(C_1-C_6 \text{ alkylene})$, $(C_1-C_6 \text{ alkylene})-Y-(C_1-C_6 \text{ alkylene})-Y-(C_1-C_6 \text{ alkylene})$, $(C_2-C_6 \text{ alkenylene})-Y-(C_2-C_6 \text{ alkenylene})$, and $(C_2-C_6 \text{ alkynylene})-Y-(C_2-C_6 \text{ alkynylene})$;

where Y is independently selected from O, S, NR^1 , aryl, and heteroaryl;

where alkylene, alkenylene, aryl, and heteroaryl are independently and optionally substituted with F, OH, $O(C_1-C_6 \text{ alkyl})$, NH_2 , $NHCH_3$, $N(CH_3)_2$, $OP(O)_3H_2$, and C_1-C_6 alkyl, where alkyl is optionally substituted with one or more F;

or alkylene, alkenylene, aryl, and heteroaryl are independently and optionally substituted with a bond to L;

D' is a drug moiety selected from:



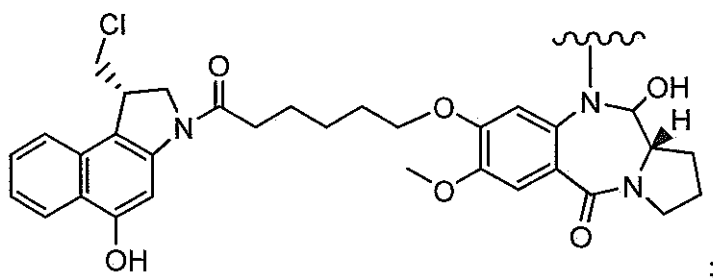
where the wavy line indicates the site of attachment to T;

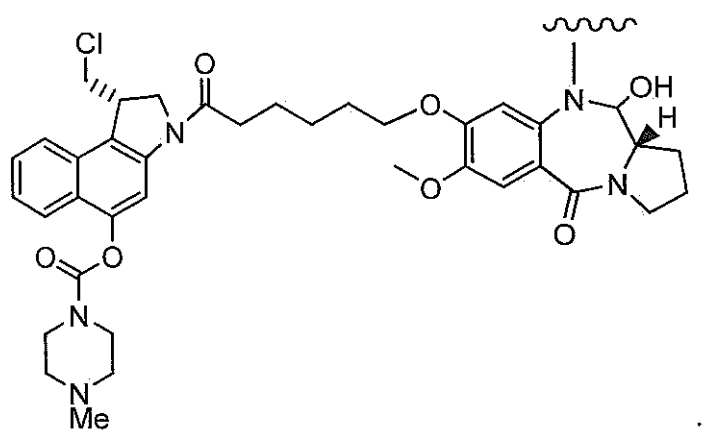
X¹ and X² are independently selected from O and NR³, where R³ is selected from H and C₁–C₆ alkyl optionally substituted with one or more F;

R⁴ is H, CO₂R, or a bond to a linker (L), where R is C₁–C₆ alkyl or benzyl; and

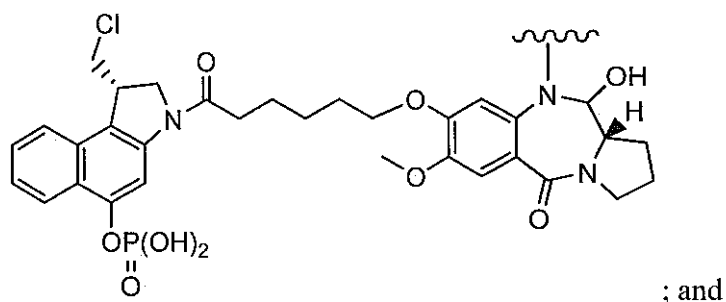
R⁵ is H or C₁–C₆ alkyl.

35. The immunoconjugate of claim 34, wherein the cytotoxic agent has a structure selected from:

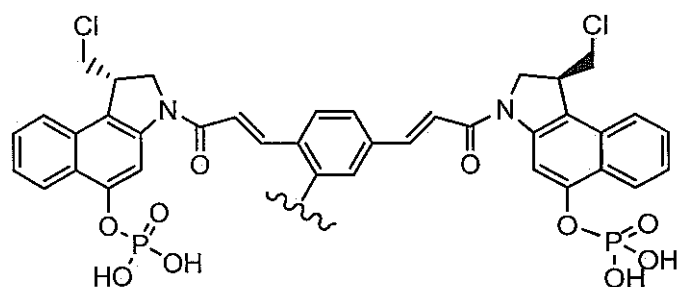




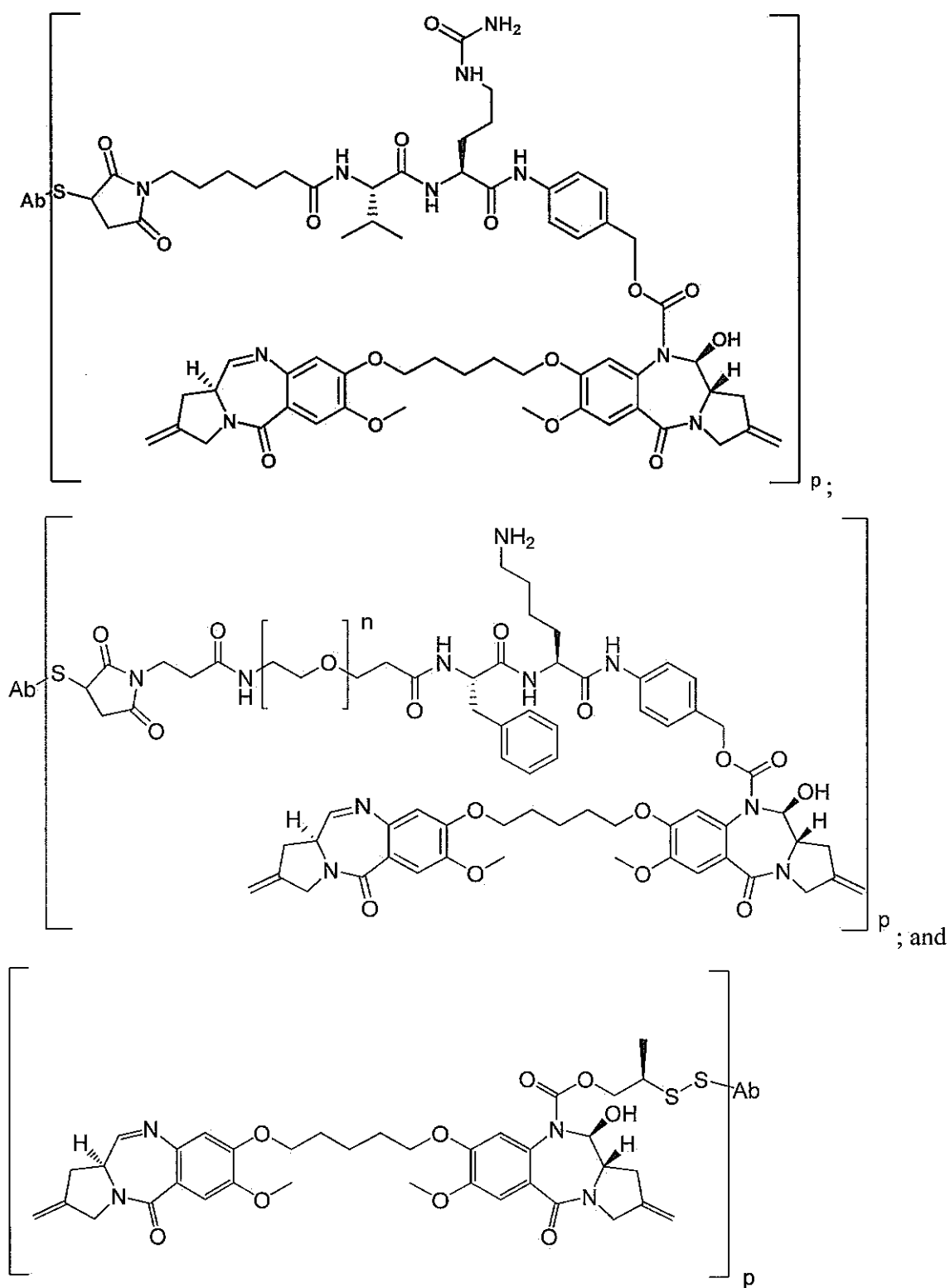
;



; and



36. The immunoconjugate of any one of claims 25 to 35, wherein the linker is cleavable by a protease.
37. The immunoconjugate of any one of claims 25 to 35, wherein the linker is acid-labile.
38. The immunoconjugate of claim 37, wherein the linker comprises hydrazone.
39. The immunoconjugate of any one of claims 25 to 35, wherein the linker comprises a disulfide.
40. The immunoconjugate of any one of claims 25 to 27 and 30 having a structure selected from:

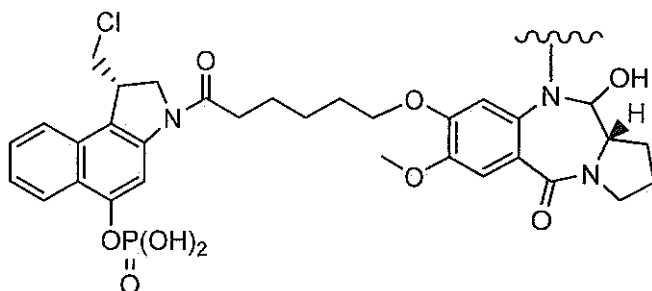


41. The immunoconjugate of any one of claims 25 to 27 and 32 having a formula selected from:



42.

43. The immunoconjugate of any one of claims 24 to 27, wherein the cytotoxic agent comprises the structure:



44. The immunoconjugate of any one of claims 25 to 43, wherein p ranges from 1.3-2 or from 2-5.
45. A pharmaceutical formulation comprising the immunoconjugate of any one of claims 24 to 44 and a pharmaceutically acceptable carrier.
46. The pharmaceutical formulation of claim 45, further comprising an additional therapeutic agent.
47. The pharmaceutical formulation of claim 46, wherein the additional therapeutic agent is an antibody or immunoconjugate that binds HER2.
48. The pharmaceutical formulation of claim 47, wherein the additional therapeutic agent is (i) an antibody or immunoconjugate that binds to domain II of HER2, and/or (ii) an antibody or immunoconjugate that binds to domain IV or HER2.
49. The pharmaceutical formulation of claim 47, wherein the additional therapeutic agent is (i) an antibody or immunoconjugate that binds to epitope 2C4, and/or (ii) an antibody or immunoconjugate that binds to epitope 4D5.
50. The pharmaceutical formulation of claim 46, wherein the additional therapeutic agent is selected from trastuzumab, trastuzumab-MCC-DM1 (T-DM1), and pertuzumab.
51. The pharmaceutical formulation of claim 46, further comprising (1) trastuzumab or T-DM1, and (2) pertuzumab.
52. Use of the immunoconjugate of any one of claims 24 to 44 in the manufacture of a medicament for treating an individual having a HER2-positive cancer.
53. The use of claim 52, wherein the HER2-positive cancer is breast cancer or gastric cancer.
54. The use of claim 53, wherein the HER2-positive breast cancer is early-stage breast cancer.
55. The use of claim 53, wherein the HER2-positive breast cancer is metastatic breast cancer.
56. The use of any one of claims 52 to 55, wherein the medicament is to be administered with an additional therapeutic agent to the individual.
57. Use of the pharmaceutical formulation of any one of claims 45 to 51 in the manufacture of a medicament for treating an individual having a HER2-positive cancer.
58. The use of claim 57, wherein the HER2-positive cancer is breast cancer or gastric cancer.
59. The use of claim 58, wherein the HER2-positive breast cancer is early-stage breast cancer.
60. The use of claim 58, wherein the HER2-positive breast cancer is metastatic breast cancer.
61. The use of any one of claims 57 to 60, wherein the medicament is to be administered with an additional therapeutic agent to the individual.

62. The use of claim 56, wherein the additional therapeutic agent is an antibody or immunoconjugate that binds HER2.
63. The use of claim 62, wherein the additional therapeutic agent is (i) an antibody or immunoconjugate that binds to domain II of HER2, and/or (ii) an antibody or immunoconjugate that binds to domain IV or HER2.
64. The use of claim 62, wherein the additional therapeutic agent is (i) an antibody or immunoconjugate that binds to epitope 2C4, and/or (ii) an antibody or immunoconjugate that binds to epitope 4D5.
65. The use of claim 56, wherein the additional therapeutic agent is selected from trastuzumab, trastuzumab-MCC-DM1 (T-DM1), and pertuzumab.
66. The use of claim 56, wherein the additional therapeutic agents are selected from (1) trastuzumab, (2) T-DM1, (3) pertuzumab, (4) trastuzumab and pertuzumab, (5) a chemotherapeutic agent, and (6) trastuzumab, pertuzumab, and a chemotherapeutic agent.
67. The use of claim 56, wherein the additional therapeutic agent comprises a chemotherapeutic agent.
68. The use of claim 62, wherein the additional therapeutic agent further comprises a chemotherapeutic agent.
69. The use of any one of claims 66 to 68, wherein the chemotherapeutic agent is selected from doxorubicin, cyclophosphamide, paclitaxel, and docetaxel.
70. The use of any one of claims 56 and 62 to 69, wherein the HER2-positive cancer is breast cancer or gastric cancer.
71. The use of claim 70, wherein the HER2-positive breast cancer is metastatic breast cancer.
72. The use of claim 70, wherein the HER2-positive breast cancer is early-stage breast cancer.
73. The use of any one of claims 52 to 72, wherein the HER2-positive cancer is recurrent cancer.
74. The use of claim 73, wherein the recurrent cancer is locally recurrent cancer.
75. The use of any one of claims 52 to 71, wherein the HER2-positive cancer is advanced cancer.
76. The use of any one of claims 52 to 75, wherein the HER2-positive cancer is non-resectable.
77. Use of the immunoconjugate of any one of claims 24 to 44 in the manufacture of a medicament for treating an individual having a HER2-positive cancer, wherein the immunoconjugate of any one of claims 24 to 44 is to be administered to the individual as neoadjuvant treatment, and subsequently as adjuvant treatment after said cancer has been removed by definitive surgery.
78. The use of claim 77, wherein the HER2-positive cancer is breast cancer or gastric cancer.
79. The use of claim 78, wherein the HER2-positive cancer is breast cancer.
80. The use of the pharmaceutical formulation of any one of claims 45 to 51 in the manufacture of a medicament for treating an individual having a HER2-positive cancer, wherein the pharmaceutical formulation of any one of claims 45 to 51 is to be administered to the individual as neoadjuvant treatment, and subsequently as adjuvant treatment after said cancer has been removed by definitive surgery.
81. The use of claim 80, wherein the HER2-positive cancer is breast cancer or gastric cancer.
82. The use of claim 81, wherein the HER2-positive cancer is breast cancer.
83. An *in vitro* method of inhibiting proliferation of a HER2-positive cell, the method comprising exposing the cell to the immunoconjugate of any one of claims 24 to 44 under conditions permissive for binding of the immunoconjugate to HER2 on the surface of the cell, thereby inhibiting proliferation of the cell.

84. The method of claim 83, wherein the cell is a breast cancer cell or a gastric cancer cell.
85. The antibody of any one of claims 1 to 20 conjugated to a label.
86. The antibody of claim 85, wherein the label is a positron emitter.
87. The antibody of claim 86, wherein the positron emitter is ^{89}Zr .
88. An *in vitro* method of detecting human HER2 in a biological sample comprising contacting the biological sample with the anti-HER2 antibody of any one of claims 1 to 20 and 85 to 87 under conditions permissive for binding of the anti-HER2 antibody to a naturally occurring human HER2, and detecting whether a complex is formed between the anti-HER2 antibody and a naturally occurring human HER2 in the biological sample.
89. The method of claim 88, wherein the biological sample is a breast cancer or gastric cancer sample.
90. The anti-HER2 antibody of any one of claims 1 to 20 for use in detecting a HER2-positive cancer in a subject having or suspected of having a HER2-positive cancer.
91. The anti-HER2 antibody of claim 90, wherein the anti-HER2 antibody is conjugated to a label, wherein the label is a positron emitter.
92. The anti-HER2 antibody of claim 91, wherein the positron emitter is ^{89}Zr .