

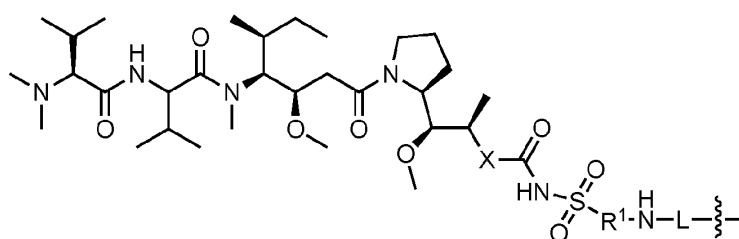
**WE CLAIM:**

1. An antibody-drug conjugate comprising an anti-HER2 biparatopic antibody conjugated to an auristatin analogue via a linker (L) at a low average drug-to-antibody ratio (DAR),

wherein the anti-HER2 biparatopic antibody comprises a first antigen-binding polypeptide construct which binds a first HER2 epitope and a second antigen-binding polypeptide construct which binds a second HER2 epitope, the first and second HER2 epitopes being different epitopes, and

wherein the low average DAR is an average DAR of less than 3.9.

2. The antibody-drug conjugate according to Claim 1, wherein the auristatin analogue-linker has general Formula (II):

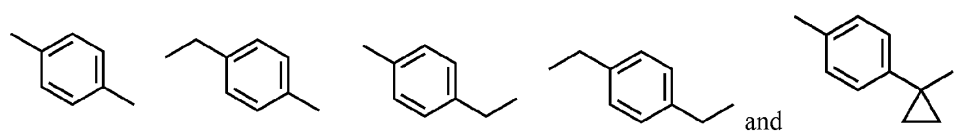


(II)

wherein:

X is  $-\text{C}(\text{O})\text{NHCH}(\text{CH}_2\text{R}^2)-$ , or X is absent;

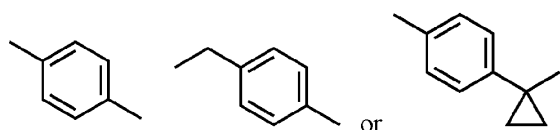
$\text{R}^1$  is selected from:



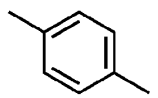
L is the linker, and

⋯ represents the point of attachment of the auristatin analogue-linker to the anti-HER2 biparatopic antibody.

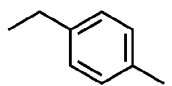
3. The antibody-drug conjugate according to Claim 2, wherein  $\text{R}^1$  is:



4. The antibody-drug conjugate according to Claim 2, wherein X is absent.
5. The antibody-drug conjugate according to Claim 4, wherein R<sup>1</sup> is:

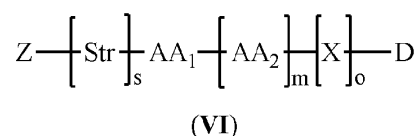


6. The antibody-drug conjugate according to Claim 2, wherein X is -C(O)NHCH(CH<sub>2</sub>R<sup>2</sup>)-.
7. The antibody-drug conjugate according to Claim 6, wherein R<sup>1</sup> is:



8. The antibody-drug conjugate according to any one of Claims 1 to 7, wherein the average DAR is between 0.5 and 3.5.
9. The antibody-drug conjugate according to any one of Claims 1 to 7, wherein the average DAR is between 0.5 and 2.5.
10. The antibody-drug conjugate according to any one of Claims 1 to 9, wherein the conjugate comprises 5% or more DAR0 species.
11. The antibody-drug conjugate according to any one of Claims 1 to 9, wherein the conjugate comprises 15% or more DAR0 species.
12. The antibody-drug conjugate according to any one of Claims 1 to 9, wherein the conjugate comprises between about 5% and about 50% DAR0 species.
13. The antibody-drug conjugate according to any one of Claims 1 to 9, wherein the conjugate comprises between about 10% and about 30% DAR0 species.

14. The antibody-drug conjugate according to any one of Claims 1 to 9, wherein the conjugate comprises between about 10% and about 25% DAR0 species.
15. The antibody-drug conjugate according to any one of Claims 1 to 9, wherein the conjugate comprises between about 15% and about 25% DAR0 species.
16. The antibody-drug conjugate according to any one of Claims 1 to 15, wherein the conjugate comprises 25% or less DAR6 or greater species.
17. The antibody-drug conjugate according to any one of Claims 1 to 15, wherein the conjugate comprises 15% or less DAR6 or greater species.
18. The antibody-drug conjugate according to any one of Claims 1 to 15, wherein the conjugate comprises between 0% and about 15% DAR6 or greater species.
19. The antibody-drug conjugate according to any one of Claims 1 to 15, wherein the conjugate comprises between about 0% and about 10% DAR6 or greater species.
20. The antibody-drug conjugate according to any one of Claims 1 to 19, wherein L is a cleavable linker.
21. The antibody-drug conjugate according to Claim 20, wherein L is a protease-cleavable linker.
22. The antibody-drug conjugate according to any one of Claims 1 to 21, wherein L has general Formula (VI):



wherein:

Z is a functional group capable of reacting with the target group on the anti-HER2 biparatopic antibody;

Str is a stretcher;

AA<sub>1</sub> and AA<sub>2</sub> are each independently an amino acid, wherein AA<sub>1</sub>-[AA<sub>2</sub>]<sub>m</sub> forms a protease cleavage site;

X is a self-immolative group;

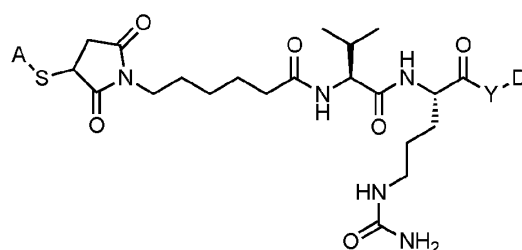
D is the point of attachment to the auristatin analogue;

s is 0 or 1;

m is an integer between 1 and 4, and

o is 0, 1 or 2.

23. The antibody-drug conjugate according to any one of Claims 1 to 21, wherein L has general Formula (VIII):



(VIII)

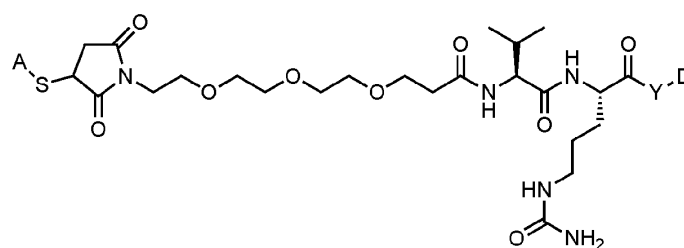
wherein:

A-S- is the point of attachment to the anti-HER2 biparatopic antibody;

Y is one or more additional linker components, or is absent, and

D is the point of attachment to the auristatin analogue.

24. The antibody-drug conjugate according to any one of Claims 1 to 21, wherein L has general Formula (IX):



(IX)

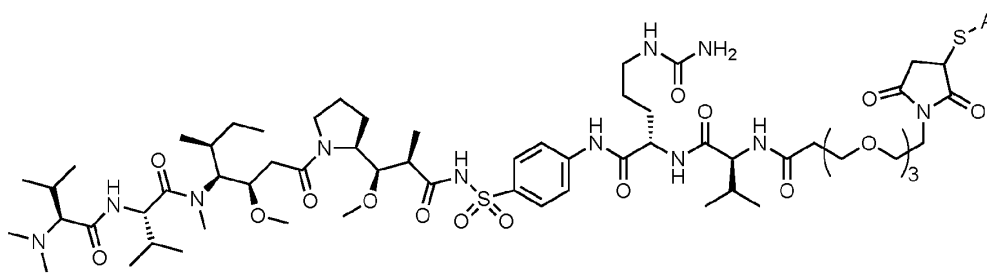
wherein:

A-S- is the point of attachment to the anti-HER2 biparatopic antibody;

Y is one or more additional linker components, or is absent, and

D is the point of attachment to the auristatin analogue.

25. The antibody-drug conjugate according to Claim 1, wherein the auristatin analogue-linker has the structure:



wherein A-S- is the point of attachment to the anti-HER2 biparatopic antibody.

26. The antibody-drug conjugate according to Claim 25, wherein the average DAR is between 0.5 and 2.5.
27. The antibody-drug conjugate according to Claim 25 or 26, wherein the conjugate comprises 5% or more DAR0 species.
28. The antibody-drug conjugate according to Claim 25 or 26, wherein the conjugate comprises between about 5% and about 50% DAR0 species.
29. The antibody-drug conjugate according to Claim 25 or 26, wherein the conjugate comprises 25% or less DAR6 or greater species.
30. The antibody-drug conjugate according to Claim 25 or 26, wherein the conjugate comprises between 0% and about 15% DAR6 or greater species.
31. The antibody-drug conjugate according to any one of Claims 1 to 30, wherein the anti-HER2 biparatopic antibody binds to HER2 with higher affinity compared to a corresponding bivalent monospecific antibody.

32. The antibody-drug conjugate according to any one of Claims 1 to 31, wherein the anti-HER2 biparatopic antibody shows a higher internalization into HER2-expressing cells compared to a corresponding bivalent monospecific antibody.
33. The antibody-drug conjugate according to any one of Claims 1 to 32, wherein the first and second antigen-binding polypeptide constructs are independently an scFv, a Fab, a Fab' or an sdAb.
34. The antibody-drug conjugate according to Claim 33, wherein the first and second antigen-binding polypeptide constructs are independently an scFv or a Fab.
35. The antibody-drug conjugate according to Claim 34, wherein the first antigen-binding polypeptide construct is an scFv, and the second antigen-binding polypeptide construct is a Fab.
36. The antibody-drug conjugate according to any one of Claims 1 to 35, wherein the first and second HER2 epitopes are non-overlapping epitopes.
37. The antibody-drug conjugate according to any one of Claims 1 to 36, wherein the first and second HER2 epitopes are on different domains of HER2.
38. The antibody-drug conjugate according to Claim 37, wherein the first HER2 epitope is on ECD4 of HER2 and the second HER2 epitope is on ECD2 of HER2.
39. The antibody-drug conjugate according to any one of Claims 1 to 38, wherein the first antigen-binding polypeptide construct competes with trastuzumab for binding to HER2.
40. The antibody-drug conjugate according to any one of Claims 1 to 39, wherein the second antigen-binding polypeptide construct competes with pertuzumab for binding to HER2.
41. The antibody-drug conjugate according to any one of Claims 1 to 38, wherein the first antigen-binding polypeptide construct comprises the CDR sequences from the ECD4-binding arm of any one of v5019, v5020, v7091, v10000, v6902, v6903 or v6717.

42. The antibody-drug conjugate according to any one of Claims 1 to 38 and 41, wherein the second antigen-binding polypeptide construct comprises the CDR sequences from the ECD2-binding arm of any one of v5019, v5020, v7091, v10000, v6902, v6903, v6717, v7133, v15079, v15080, v15081, v15082, v15083, v15084 or v15085.
43. The antibody-drug conjugate according to any one of Claims 1 to 38 and 41, wherein the second antigen-binding polypeptide construct comprises the CDR sequences from the ECD2-binding arm of v10000.
44. The antibody-drug conjugate according to any one of Claims 1 to 38, wherein the first antigen-binding polypeptide construct comprises the CDR sequences from the ECD4-binding arm of v10000, and the second antigen-binding polypeptide construct comprises the CDR sequences from the ECD2-binding arm of v10000.
45. The antibody-drug conjugate according to any one of Claims 1 to 38, wherein the first antigen-binding polypeptide construct comprises a set of six CDRs that have 90% or greater sequence identity to a set of CDRs from the ECD4-binding arm of v10000, wherein the antigen-binding polypeptide construct retains the ability to bind ECD4.
46. The antibody-drug conjugate according to any one of Claims 1 to 38 and 45, wherein the second antigen-binding polypeptide construct comprises a set of six CDRs that have 90% or greater sequence identity to a set of CDRs from the ECD2-binding arm of v10000, wherein the antigen-binding polypeptide construct retains the ability to bind ECD2.
47. The antibody-drug conjugate according to any one of Claims 1 to 38, wherein the first antigen-binding polypeptide construct comprises the CDR sequences set forth in SEQ ID NOs: 27, 28, 29, 39, 40 and 41, and the second antigen-binding polypeptide construct comprises the CDR sequences set forth in SEQ ID NOs: 67, 68, 69, 70, 71 and 72.
48. The antibody-drug conjugate of any one of Claims 1 to 47, wherein the anti-HER2 biparatopic antibody further comprises a scaffold, and wherein the first and second antigen-binding polypeptide constructs are operably linked to the scaffold.

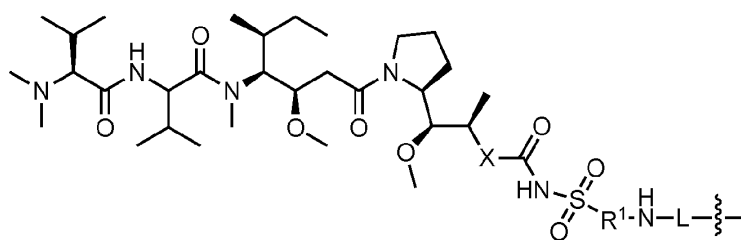
49. The antibody-drug conjugate according to Claim 48, wherein the scaffold is an IgG Fc region.
50. The antibody-drug conjugate according to Claim 49, wherein the IgG Fc region is a heterodimeric Fc region comprising a modified CH3 domain.
51. The antibody-drug conjugate according to Claim 49, wherein the IgG Fc region is a heterodimeric Fc region comprising a modified CH3 domain having a first polypeptide sequence that comprises amino acid modifications at positions F405 and Y407, and optionally further comprises an amino acid modification at position L351, and a second polypeptide sequence that comprises amino acid modifications at positions T366 and T394, and optionally further comprises an amino acid modification at position K392, wherein the amino acid modification at position F405 is F405A, F405S, F405T or F405V; the amino acid modification at position Y407 is Y407I or Y407V; the amino acid modification at position T366 is T366I, T366L or T366M; the amino acid modification at position T394 is T394W; the amino acid modification at position L351 is L351Y, and the amino acid modification at position K392 is K392F, K392L or K392M.
52. The antibody-drug conjugate according to Claim 51, wherein:
- (a) the first polypeptide sequence of the modified CH3 domain comprises the amino acid modifications L351Y, F405A and Y407V, and the second polypeptide sequence of the modified CH3 domain comprises the amino acid modifications T366L, K392M and T394W; or
- (b) the first polypeptide sequence of the modified CH3 domain comprises the amino acid modifications L351Y, F405A and Y407V, and the second polypeptide sequence of the modified CH3 domain comprises the amino acid modifications T366L, K392L and T394W; or
- (c) the first polypeptide sequence of the modified CH3 domain comprises the amino acid modifications T350V, L351Y, F405A and Y407V, and the second polypeptide sequence of the modified CH3 domain comprises the amino acid modifications T350V, T366L, K392M and T394W; or

(d) the first polypeptide sequence of the modified CH3 domain comprises the amino acid modifications T350V, L351Y, F405A and Y407V, and the second polypeptide sequence of the modified CH3 domain comprises the amino acid modifications T350V, T366L, K392L and T394W; or

(e) the first polypeptide sequence of the modified CH3 domain comprises the amino acid modifications T350V, L351Y, S400E, F405A and Y407V, and the second polypeptide sequence of the modified CH3 domain comprises the amino acid modifications T350V, T366L, N390R, K392M and T394W.

53. A pharmaceutical composition comprising the antibody-drug conjugate according to any one of Claims 1 to 52 and a pharmaceutically acceptable carrier or diluent.
54. A method of inhibiting the growth of a HER2-expressing cancer cell, the method comprising contacting the cancer cell with an effective amount of the antibody-drug conjugate according to any one of Claims 1 to 52.
55. A method of treating a HER2-expressing cancer comprising administering to a subject having a HER2-expressing cancer an effective amount of the antibody-drug conjugate according to any one of Claims 1 to 52.
56. The method according to Claim 55, wherein the HER2-expressing cancer is a breast cancer, ovarian cancer, lung cancer or gastric cancer.
57. The method according to Claim 55, wherein the HER2-expressing cancer is a breast cancer.
58. The method according to Claim 55, wherein the HER2-expressing cancer is an ovarian cancer.
59. The method according to any one of Claims 55 to 58, wherein the HER2-expressing cancer is scored as HER2 negative by immunohistochemistry.
60. An antibody-drug conjugate according to any one of Claims 1 to 52 for use in therapy.

61. An antibody-drug conjugate according to any one of Claims 1 to 52 for use to treat a HER2-expressing cancer in a subject in need thereof.
62. The antibody-drug conjugate for use according to Claim 61, wherein the HER2-expressing cancer is a breast cancer, ovarian cancer, lung cancer or gastric cancer.
63. The antibody-drug conjugate for use according to Claim 61, wherein the HER2-expressing cancer is a breast cancer.
64. The antibody-drug conjugate for use according to Claim 61, wherein the HER2-expressing cancer is an ovarian cancer.
65. The antibody-drug conjugate for use according to any one of Claims 61 to 64, wherein the HER2-expressing cancer is scored as HER2 negative by immunohistochemistry.
66. Use of the antibody-drug conjugate according to any one of Claims 1 to 52 in the manufacture of a medicament for the treatment of a HER2-expressing cancer.
67. The use according to Claim 66, wherein the HER2-expressing cancer is a breast cancer, ovarian cancer, lung cancer or gastric cancer.
68. The use according to Claim 66, wherein the HER2-expressing cancer is a breast cancer.
69. The use according to Claim 66, wherein the HER2-expressing cancer is an ovarian cancer.
70. The use according to any one of Claims 66 to 69, wherein the HER2-expressing cancer is scored as HER2 negative by immunohistochemistry.
71. An antibody-drug conjugate composition comprising an anti-HER2 biparatopic antibody conjugated to an auristatin analogue via a linker (L), the auristatin analogue-linker having general Formula (II):

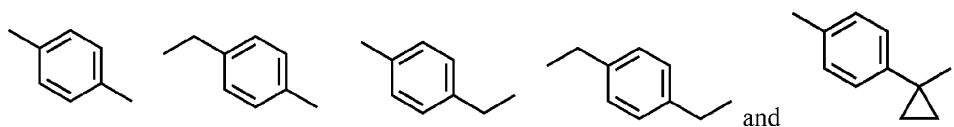


(II)

wherein:

X is absent;

R<sup>1</sup> is selected from:



L is the linker, and

⋈ represents the point of attachment of the auristatin analogue-linker to the anti-HER2 biparatopic antibody;

wherein the anti-HER2 biparatopic antibody comprises a first antigen-binding polypeptide construct which binds a first HER2 epitope on ECD4 of HER2 and a second antigen-binding polypeptide construct which binds a second HER2 epitope on ECD2 of HER2, and

wherein the antibody-drug conjugate composition has an average DAR of between 0.5 and 2.5 and comprises between about 10% and about 30% DAR0 species and between 0% and about 15% DAR6 or greater species.