[0007e] In a further aspect of the present disclosure, there is provided a use of the antibody-drug conjugate disclosed herein in the manufacture of a medicament for the treatment of a HER2-expressing cancer.

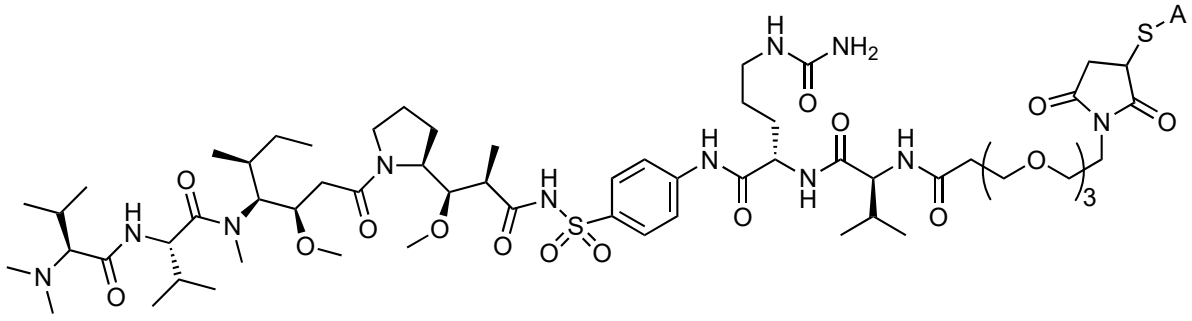
[0007f] In another aspect of the present disclosure, there is provided a composition comprising a plurality of an antibody-drug conjugate comprising an anti-HER2 biparatopic antibody conjugated to an auristatin analogue via a linker,

the anti-HER2 biparatopic antibody comprising:

(i) a first antigen-binding polypeptide construct comprising the CDR sequences as set forth in SEQ ID NOs: 27, 28, 29, 39, 40 and 41, wherein the first antigen-binding polypeptide construct is a Fab, and

(ii) a second antigen-binding polypeptide construct comprising the CDR sequences as set forth in SEQ ID NOs: 67, 68, 69, 70, 71 and 72, wherein the second antigen-binding polypeptide construct is an scFv,

the auristatin analogue and linker having the structure:



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

wherein the composition has an average drug-to-antibody ratio (DAR) of between 1.8 and 2.5 and the composition comprises between 10% and 25% DAR0 species of the antibody-drug conjugate.

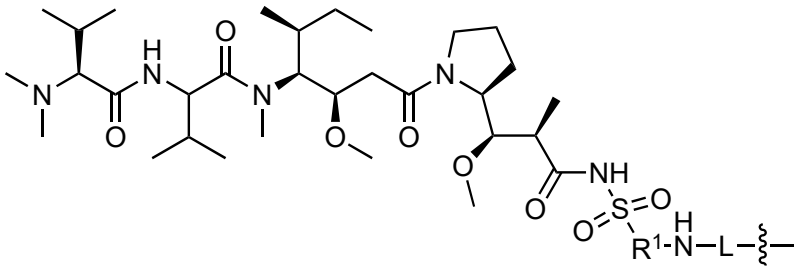
[0008] In certain examples of the antibody-drug conjugate the auristatin analogue-linker has general Formula (**II**):

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, wherein the first and second HER2 epitopes are on different domains of HER2,

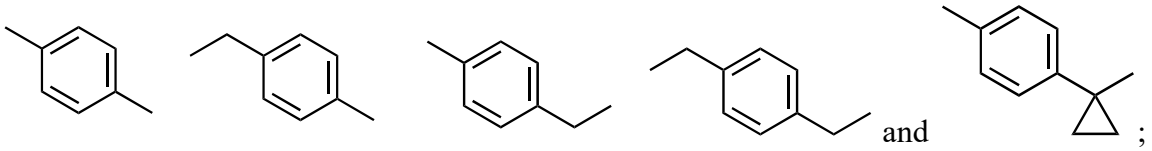
wherein the auristatin analogue and linker have general Formula (X):



(X)

wherein:

R^1 is selected from:



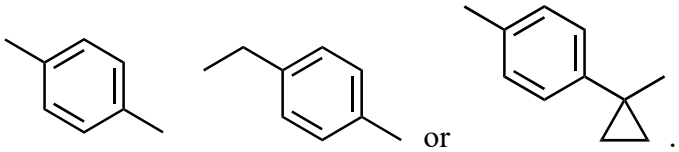
and ;

L is the linker, and

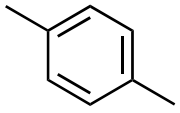
≈ represents the point of attachment of the linker to the anti-HER2 biparatopic antibody, and

wherein the low average DAR is an average DAR between 1.5 and 2.5.

2. The antibody-drug conjugate according to Claim 1, wherein R^1 is:



3. The antibody-drug conjugate according to Claim 1, wherein R^1 is:



4. The antibody-drug conjugate according to any one of Claims 1 to 3, wherein the average DAR is between 1.8 and 2.5.

5. The antibody-drug conjugate according to any one of Claims 1 to 3, wherein the average DAR is about 2.0.

6. The antibody-drug conjugate according to any one of Claims 1 to 5, wherein the conjugate comprises between 10% and 30% DAR0 species.

7. The antibody-drug conjugate according to any one of Claims 1 to 5, wherein the conjugate comprises between 10% and 25% DAR0 species.

8. The antibody-drug conjugate according to any one of Claims 1 to 5, wherein the conjugate comprises between 15% and 25% DAR0 species.

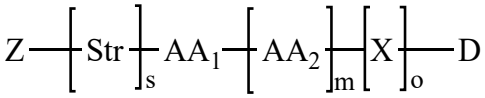
9. The antibody-drug conjugate according to any one of Claims 1 to 8, wherein the conjugate comprises between 0% and 15% DAR6 or greater species.

10. The antibody-drug conjugate according to any one of Claims 1 to 8, wherein the conjugate comprises between 0% and 10% DAR6 or greater species.

11. The antibody-drug conjugate according to any one of Claims 1 to 10, wherein L is a cleavable linker.

12. The antibody-drug conjugate according to Claim 11, wherein L is a protease-cleavable linker.

13. The antibody-drug conjugate according to any one of Claims 1 to 12, wherein L has general Formula (**VI**):



(**VI**)

wherein:

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

Str is a stretcher;

AA_1 and AA_2 are each independently an amino acid, wherein AA_1-$[AA_2]_m$ forms a protease cleavage site;

X is a self-immolative group;

D is the point of attachment to the auristatin analogue;

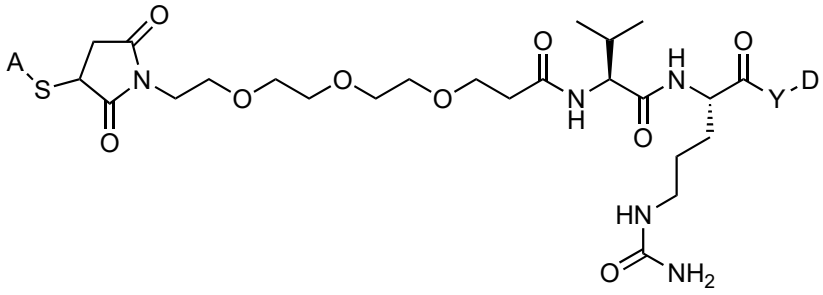
s is 0 or 1;

m is an integer between 1 and 4, and

o is 0, 1 or 2.

14. The antibody-drug conjugate according to Claim 13, wherein in Formula (VI), s is 1 and o is 0.

15. The antibody-drug conjugate according to any one of Claims 1 to 12, wherein L has general Formula (**IX**):



(**IX**)

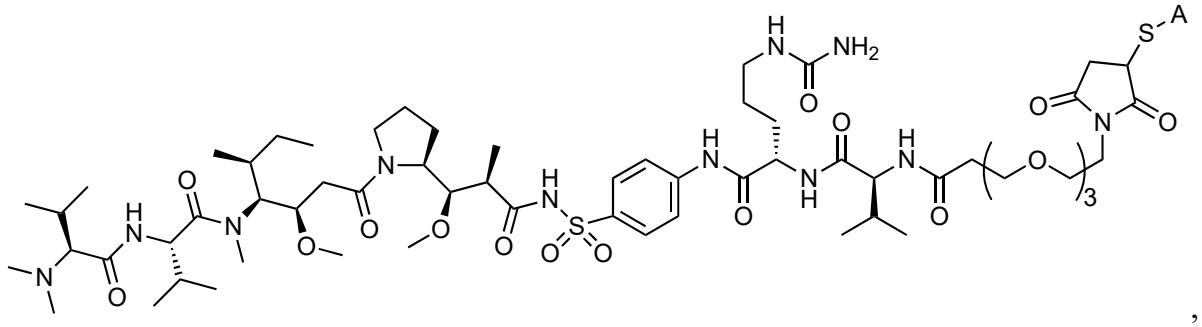
wherein:

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.

16. The antibody-drug conjugate according to any one of Claims 1 to 10, wherein the auristatin analogue and linker have the structure:



,

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

17. The antibody-drug conjugate according to any one of Claims 1 to 16, wherein the anti-HER2 biparatopic antibody binds to HER2 with higher affinity compared to a corresponding bivalent monospecific antibody.

18. The antibody-drug conjugate according to any one of Claims 1 to 17, wherein the anti-HER2 biparatopic antibody shows a higher internalization into HER2-expressing cells compared to a corresponding bivalent monospecific antibody.

19. The antibody-drug conjugate according to any one of Claims 1 to 18, wherein the first and second antigen-binding polypeptide constructs are independently an scFv or a Fab.

20. The antibody-drug conjugate according to Claim 19, wherein the first antigen-binding polypeptide construct is an scFv, and the second antigen-binding polypeptide construct is a Fab.

21. The antibody-drug conjugate according to any one of Claims 1 to 20, wherein the first HER2 epitope is on ECD4 of HER2 and the second HER2 epitope is on ECD2 of HER2.

22. The antibody-drug conjugate according to any one of Claims 1 to 21, wherein the first antigen-binding polypeptide construct competes with trastuzumab for binding to HER2.

23. The antibody-drug conjugate according to any one of Claims 1 to 22, wherein the second antigen-binding polypeptide construct competes with pertuzumab for binding to HER2.

24. The antibody-drug conjugate according to any one of Claims 1 to 21, 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, and 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.

25. The antibody-drug conjugate according to any one of Claims 1 to 21, 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.

26. The antibody-drug conjugate according to any one of Claims 1 to 20, 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.

27. The antibody-drug conjugate according to any one of Claims 1 to 20, wherein:

the first antigen-binding polypeptide construct comprises the VH sequence as set forth in SEQ ID NO: 38 and the VL sequence as set forth in SEQ ID NO: 26, and

the second antigen-binding polypeptide construct comprises the VH sequence as set forth in SEQ ID NO: 66 and the VL sequence as set forth in SEQ ID NO: 65.

28. The antibody-drug conjugate according to Claim 21, wherein the first antigen-binding polypeptide construct comprises the CDR sequences set forth in SEQ ID NOs: 67, 68, 69, 70, 71 and 72, and the second antigen-binding polypeptide construct comprises the CDR sequences set forth in SEQ ID NOs: 27, 28, 29, 39, 40 and 41.

29. The antibody-drug conjugate according to Claim 21, wherein:

the first antigen-binding polypeptide construct comprises the VH sequence as set forth in SEQ ID NO: 66 and the VL sequence as set forth in SEQ ID NO: 65, and

the second antigen-binding polypeptide construct comprises the VH sequence as set forth in SEQ ID NO: 38 and the VL sequence as set forth in SEQ ID NO: 26.

30. The antibody-drug conjugate according to any one of Claims 1 to 29, wherein the anti-HER2 biparatopic antibody further comprises a scaffold that is an IgG Fc region, and wherein the first and second antigen-binding polypeptide constructs are operably linked to the scaffold.

31. The antibody-drug conjugate according to Claim 30, wherein the IgG Fc region is a heterodimeric Fc region comprising a modified CH3 domain.

32. The antibody-drug conjugate according to Claim 31, wherein the modified CH3 domain comprises a first polypeptide sequence and a second polypeptide sequence, and 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.

33. The antibody-drug conjugate according to Claim 31, wherein the modified CH3 domain comprises a first polypeptide sequence and a second polypeptide sequence, and wherein the first polypeptide sequence comprises the amino acid modifications T350V, L351Y, F405A and Y407V, and the second polypeptide sequence comprises the amino acid modifications T350V, T366L, K392L and T394W.

34. A pharmaceutical composition comprising the antibody-drug conjugate according to any one of Claims 1 to 33 and a pharmaceutically acceptable carrier or diluent.

35. An antibody-drug conjugate according to any one of Claims 1 to 33 for use in therapy.

36. An antibody-drug conjugate according to any one of Claims 1 to 33 for use to treat a HER2-expressing cancer in a subject in need thereof.

37. Use of the antibody-drug conjugate according to any one of Claims 1 to 33 in the manufacture of a medicament for the treatment of a HER2-expressing cancer.

38. The antibody-drug conjugate for use according to Claim 36, or the use according to Claim 37, wherein the HER2-expressing cancer is a breast cancer, an ovarian cancer, a lung cancer or a gastric cancer.

39. The antibody-drug conjugate for use according to Claim 36, or the use according to Claim 37, wherein the HER2-expressing cancer is a breast cancer.

40. The antibody-drug conjugate for use according to Claim 36, or the use according to Claim 37, wherein the HER2-expressing cancer is an ovarian cancer.

41. The antibody-drug conjugate for use according to any one of Claims 36 and 38 to 40, or the use according to any one of Claims 37 to 40, wherein the HER2-expressing cancer is scored as HER2 negative by immunohistochemistry.

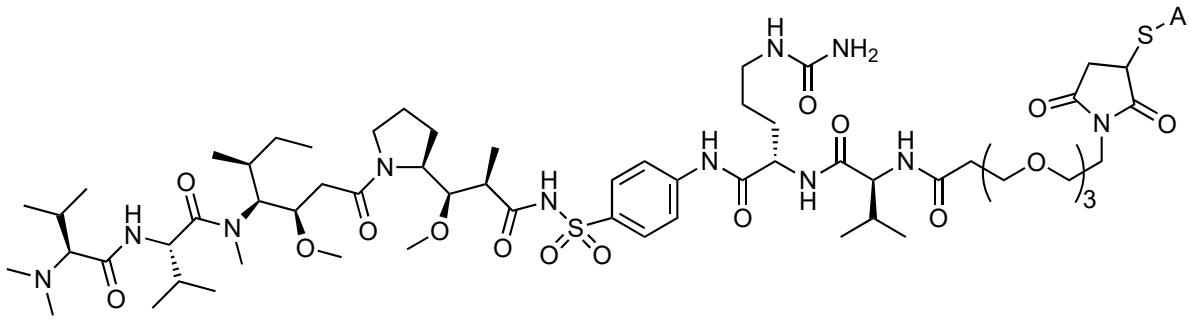
42. A composition comprising a plurality of an antibody-drug conjugate comprising an anti-HER2 biparatopic antibody conjugated to an auristatin analogue via a linker,

the anti-HER2 biparatopic antibody comprising:

(i) a first antigen-binding polypeptide construct comprising the CDR sequences as set forth in SEQ ID NOs: 27, 28, 29, 39, 40 and 41, wherein the first antigen-binding polypeptide construct is a Fab, and

(ii) a second antigen-binding polypeptide construct comprising the CDR sequences as set forth in SEQ ID NOs: 67, 68, 69, 70, 71 and 72, wherein the second antigen-binding polypeptide construct is an scFv,

the auristatin analogue and linker having the structure:



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

wherein the composition has an average drug-to-antibody ratio (DAR) of between 1.8 and 2.5 and the composition comprises between 10% and 25% DAR0 species of the antibody-drug conjugate.

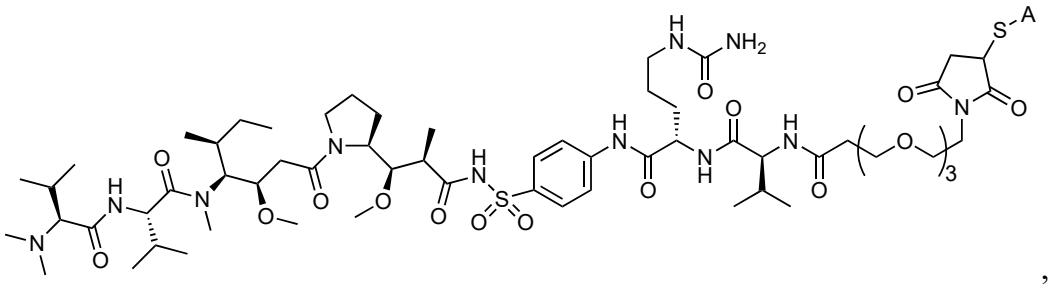
43. The antibody-drug conjugate according to Claim 1, wherein

(a) the first antigen-binding polypeptide construct of the anti-HER2 biparatopic antibody is an scFv comprising the VH sequence as set forth in SEQ ID NO: 66 and the VL sequence

as set forth in SEQ ID NO: 65, and the second antigen-binding polypeptide construct of the anti-HER2 biparatopic antibody is a Fab comprising the VH sequence as set forth in SEQ ID NO: 38 and the VL sequence as set forth in SEQ ID NO: 26;

(b) the anti-HER2 biparatopic antibody further comprises a scaffold that is a heterodimeric IgG Fc region comprising a modified CH3 domain, wherein the first and second antigen-binding polypeptide constructs are operably linked to the scaffold, wherein the modified CH3 domain comprises a first polypeptide sequence and a second polypeptide sequence, the first polypeptide sequence comprising the amino acid modifications T350V, L351Y, F405A and Y407V, and the second polypeptide sequence comprising the amino acid modifications T350V, T366L, K392L and T394W;

(c) the auristatin analogue and linker have the structure:



,

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

(d) the average DAR is between 1.8 and 2.5; and

(e) the conjugate comprises between 10% and 25% DAR0 species.