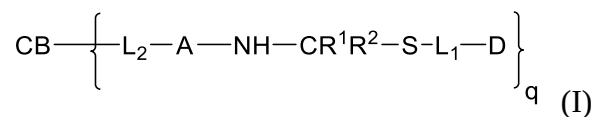


Claims

1. A biparatopic antibody or antigen binding fragment thereof that specifically binds a human folate receptor 1 (FR α), wherein the antibody or antigen-binding fragment comprises:
 - (a) a first FR α -binding domain that comprises a first variable heavy chain (VH) and a first variable light chain (VL) and that bind to a first epitope of FR α ; and
 - (b) a second FR α -binding domain that comprises a second VH and a second VL and that binds to a second epitope of FR α , whereinthe first VH comprises VH CDR1-3 comprising the amino acid sequences of (a) SEQ ID NOs: 10-12 or (b) SEQ ID NOs: 15, 16, and 12, respectively;
the first VL comprises VL CDR1-3 comprising the amino acid sequences of SEQ ID NOs: 4-6, respectively;
the second VH comprises VH CDR1-3 comprising the amino acid sequences of (a) SEQ ID NOs: 7-9 or (b) SEQ ID NOs: 13, 14, and 9, respectively; and
the second VL comprises VL CDR1-3 comprising the amino acid sequences of SEQ ID NOs: 1-3, respectively.
2. The biparatopic antibody or antigen binding fragment thereof of claim 1, wherein (i) the first VH comprises the amino acid sequence of SEQ ID NO:24 and the first VL comprises the amino acid sequence of SEQ ID NO:19 or (ii) the second VH comprises the amino acid sequence of SEQ ID NO: 23 and the second VL comprises the amino acid sequence of SEQ ID NO:18.
3. The biparatopic antibody or antigen binding fragment thereof of claim 1, wherein (i) the first VH comprises the amino acid sequence of SEQ ID NO:24 and the first VL comprises the amino acid sequence of SEQ ID NO:19 and (ii) the second VH comprises the amino acid sequence of SEQ ID NO: 23 and the second VL comprises the amino acid sequence of SEQ ID NO:18.

4. The biparatopic antibody or antigen-binding fragment thereof of any one of claims 1-3, wherein the first FR α -binding domain is a single-chain variable fragment (scFv) and the second FR α -binding domain comprises a VH and a VL on separate polypeptides.
5. The biparatopic antibody or antigen-binding fragment thereof of any one of claims 1-3, wherein the second FR α -binding domain is a single-chain variable fragment (scFv) and the first FR α -binding domain comprises a VH and a VL on separate polypeptides.
6. The biparatopic antibody or antigen-binding fragment thereof of claim 5, wherein the scFv has a peptide orientation of VH-linker-VL.
7. The biparatopic antibody or antigen-binding fragment thereof of claim 5, wherein the scFv has a peptide orientation of VL-linker-VH.
8. The biparatopic antibody or antigen binding fragment thereof of claim 6 or 7 wherein the linker is a glycine-serine linker.
9. The biparatopic antibody or antigen binding fragment thereof of any one of claims 1-8, which has a knob-in-hole (KIH) structure.
10. The biparatopic antibody or antigen binding fragment thereof of claim 9, wherein the second FR α binding domain is on the knob side of the KIH structure.
11. The biparatopic antibody or antigen binding fragment thereof of claim 9, wherein the first FR α binding domain is on the hole side of the KIH structure.
12. The biparatopic antibody or antigen binding fragment thereof of any one of claims 1-11 comprising the amino acid sequences of SEQ ID NOs: 41-43.

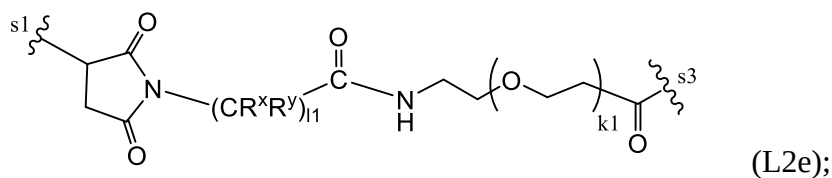
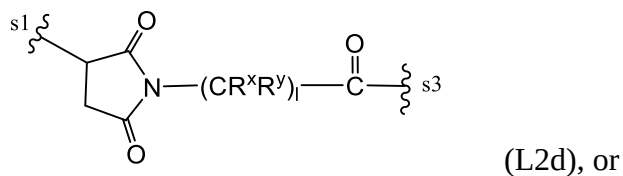
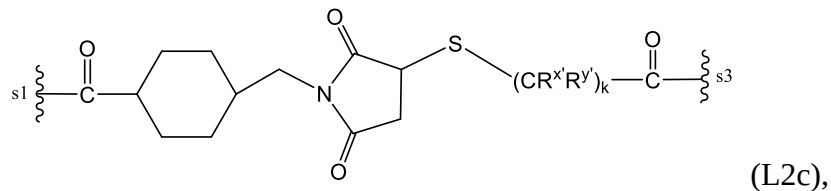
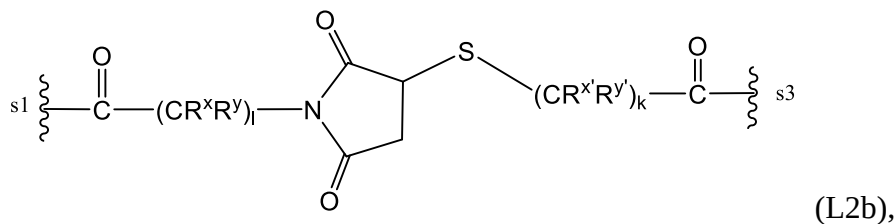
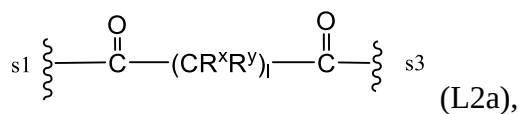
13. A combination of isolated nucleic acid molecules encoding the biparatopic antibody or antigen binding fragment thereof of claims 1-12.
14. An isolated vector comprising one of the nucleic acid molecules of claim 13.
15. A host cell comprising the combination of isolated nucleic acid molecules of claim 13 or the isolated vector of claim 14.
16. The host cell of claim 15, which is selected from the group consisting of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, yeast, CHO, YB/20, NS0, PER-C6, HEK-293T, NIH-3T3, HeLa, BHK, Hep G2, SP2/0, R1.1, B-W, L-M, COS 1, COS 7, BSC1, BSC40, BMT10 cell, plant cell, insect cell, and human cell in tissue culture.
17. A pharmaceutical composition comprising the biparatopic antibody or antigen of any one of claims 1-12, the combination of nucleic acid molecules of claim 13, the vector of claim 14, or the host cell of claim 14 or 15, and a pharmaceutically acceptable carrier or excipient.
18. A pharmaceutical composition comprising the biparatopic antibody of any one of claims 1-12, and a pharmaceutically carrier or excipient.
19. A method of making the biparatopic antibody of any one of claims 1-12 comprising (a) culturing a cell expressing said antibody; and (b) isolating the antibody from said cultured cell.
20. The method of claim 19, wherein said cell is a eukaryotic cell.
21. An immunoconjugate represented by the following formula:



or a pharmaceutically acceptable salt thereof, wherein:

CB is the biparatopic antibody or an antigen-binding fragment thereof of any one of claims 1-12;

L₂ is represented by one of the following formula:



wherein:

R^x, R^y, R^{x'} and R^{y'}, for each occurrence, are independently H, -OH, halogen, -O-(C₁₋₄ alkyl), -SO₃H, -NR₄₀R₄₁R₄₂⁺, or a C₁₋₄ alkyl optionally substituted with -OH, halogen, SO₃H or NR₄₀R₄₁R₄₂⁺, wherein R₄₀, R₄₁ and R₄₂ are each independently H or a C₁₋₄ alkyl;

l and k are each independently an integer from 1 to 10;

l₁ is an integer from 2 to 5;

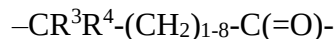
k1 is an integer from 1 to 5; and

s1 indicates the site connected to the cell-binding agent CB and s3 indicates the site connected to the A group;

A is an amino acid residue or a peptide comprising 2 to 20 amino acid residues;

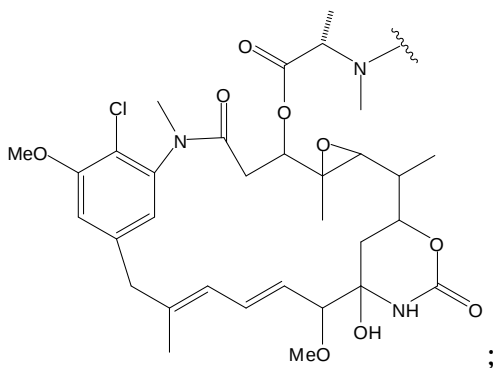
R¹ and R² are each independently H or a C₁₋₃alkyl;

L₁ is represented by the following formula:



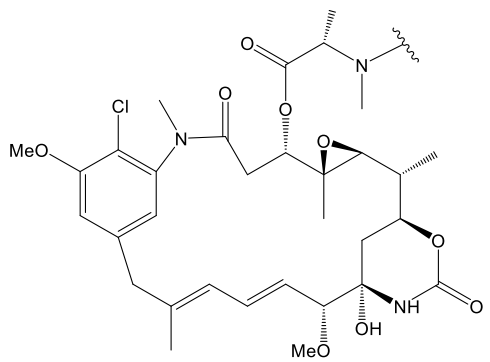
wherein R³ and R⁴ are each independently H or Me, and the $-C(=O)-$ moiety in L₁ is connected to D;

D is represented by the following formula:



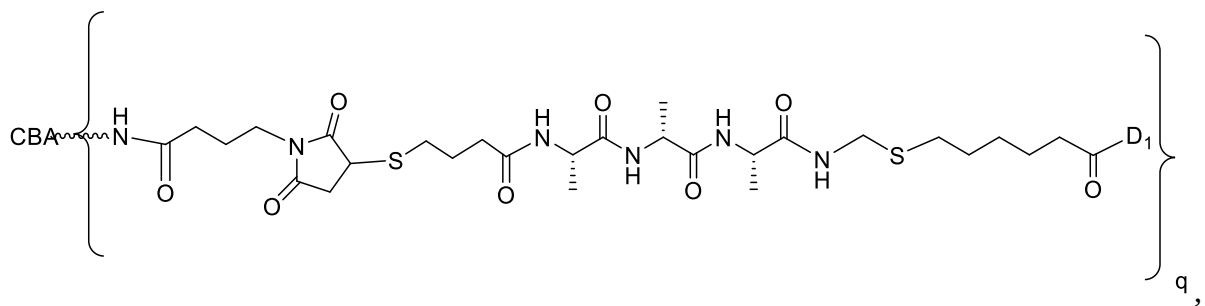
q is an integer from 1 to 20.

22. The immunoconjugate of claim 21, wherein D is represented by the following formula:



23. The immunoconjugate of claim 21, wherein the immunoconjugate is represented by the

following formula:

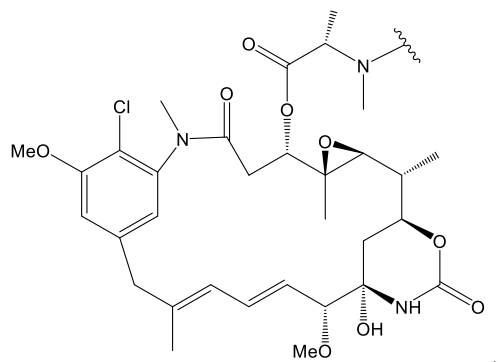


wherein:

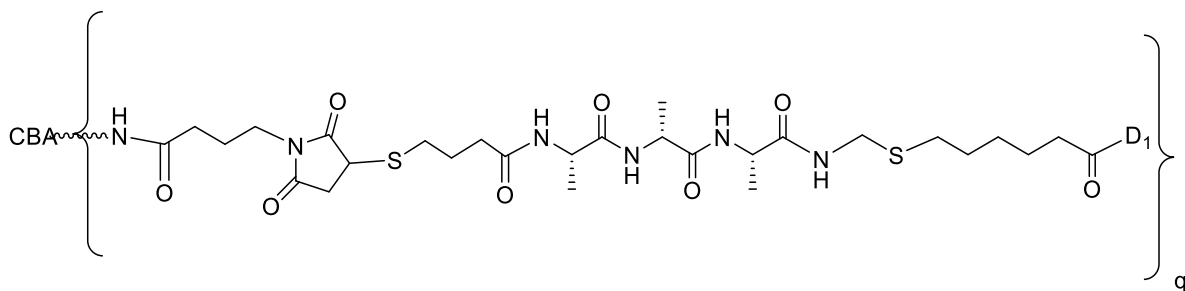
CBA is the biparatopic antibody or an antigen-binding fragment thereof of any one of claims 1-12;

q is an integer from 1 to 10;

D₁ is represented by the following formula:



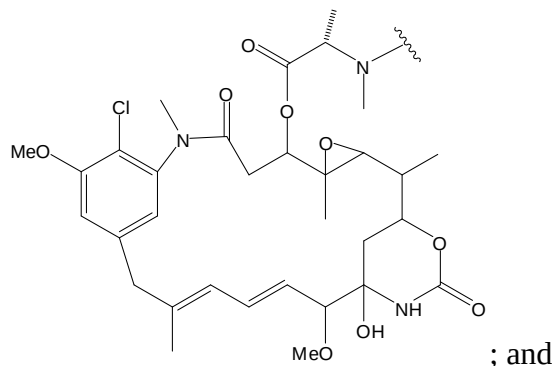
24. An immunoconjugate represented by the following formula:



or a pharmaceutically acceptable salt thereof, wherein:

CBA is a biparatopic antibody or an antigen-binding fragment comprising the amino acid sequences of SEQ ID NOS: 41-43;

D₁ is represented by the following formula:



q is an integer from 1 to 10.

25. An immunoconjugate having the formula (A) - (L) - (C), wherein:
 - (A) is a biparatopic antibody or antigen binding fragment of any one of claims 1-12;
 - (L) is a linker; and
 - (C) is a cytotoxic agent. wherein said linker (L) links (A) to (C).

26. The immunoconjugate of claim 25, wherein said linker is selected from the group consisting of N-(γ maleimidobutryloxy)sulfosuccinimide ester (sulfo-GMBS or sGMBS), γ maleimidobutyric acid N-succinimidyl ester (GMBS), N-succinimidyl 4-(2-pyridyldithio)-2-sulfobutanoate (sulfo-SPDB); N-succinimidyl 4-(2-pyridyldithio)pentanoate (SPP) or N-succinimidyl 4-(2- pyridyldithio)-2-sulfopentanoate (sulfo-SPP); N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB), N-succinimidyl 4- (maleimidomethyl) cyclohexanecarboxylate (SMCC); N-sulfosuccinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (sulfoSMCC); N-succinimidyl-4-(iodoacetyl)-aminobenzoate (SIAB); and N-succinimidyl- ester (NHS-PEG4-maleimide).

27. The immunoconjugate of claim 25 or 26, wherein the linker is sulfo-GMBS.

28. The immunoconjugate of claim 25 or 26, wherein the linker is GMBS.

29. The immunoconjugate of claim 25 or 26, wherein the linker is sulfo-SPDB.
30. The immunoconjugate of any one of claims 25-29, wherein said cytotoxic agent is selected from the group consisting of a maytansinoid, maytansinoid analog, benzodiazepine, taxoid, CC-1065, CC-1065 analog, duocarmycin, duocarmycin analog, calicheamicin, dolastatin, dolastatin analog, auristatin, tomaymycin derivative, and leptomycin derivative or a prodrug of the agent.
31. The immunoconjugate of any one of claims 25-30, wherein said cytotoxic agent is a maytansinoid.
32. The immunoconjugate of claim 31, wherein said maytansinoid is DM4.
33. The immunoconjugate of claim 31, wherein said maytansinoid is DM21.
34. A composition comprising at least one immunoconjugate of any one of claims 25-33, wherein the immunoconjugates comprise an average of 3-4 C per A.
35. A pharmaceutical composition comprising the immunoconjugate of any one of claims 21-33 and a pharmaceutically acceptable carrier.
36. The pharmaceutical composition of claim 35, wherein the pharmaceutical composition comprises an average of 3 to 4 drugs per antibody or antigen-binding fragment thereof.
37. The immunoconjugate of any one of claims 21-33 or the composition of any one of claims 34-36 for use in the treatment of cancer, wherein the cancer is ovarian cancer, uterine cancer, peritoneal cancer, fallopian tube cancer, endometrial cancer, lung cancer, or brain cancer.

38. The immunoconjugate or composition for use of claim 37, wherein the cancer is ovarian cancer.
39. The immunoconjugate or composition for use of claim 38, wherein the ovarian cancer is platinum-resistant epithelial ovarian cancer.
40. The immunoconjugate or composition for use of claim 38, wherein the ovarian cancer is relapsed epithelial ovarian cancer.
41. The immunoconjugate or composition for use of claim 38, wherein the ovarian cancer is platinum-refractory epithelial ovarian cancer.
42. The immunoconjugate or composition for use of claim 37, wherein the cancer is peritoneal cancer.
43. The immunoconjugate or composition for use of claim 37, wherein the cancer is fallopian tube cancer.
44. The immunoconjugate or composition for use of any one of claims 37-43, wherein the immunoconjugate or composition is for use with a steroid.
45. Use of the immunoconjugate of any one of claims 21-33 or the composition of any one of claims 34-36 for the manufacture of a medicament for the therapeutic treatment of cancer, wherein the cancer is ovarian cancer, uterine cancer, peritoneal cancer, fallopian tube cancer, endometrial cancer, lung cancer, or brain cancer.
46. The use of claim 45, wherein the cancer is ovarian cancer.
47. The use of claim 46, wherein the ovarian cancer is platinum-resistant epithelial ovarian cancer.
48. The use of claim 46, wherein the ovarian cancer is relapsed epithelial ovarian cancer.
49. The use of claim 46, wherein the ovarian cancer is platinum-refractory epithelial ovarian cancer.

50. The use of claim 45, wherein the cancer is uterine cancer.
51. The use of claim 45, wherein the cancer is peritoneal cancer.
52. The use of claim 45, wherein the cancer is fallopian tube cancer.
53. The use of any one of claims claim 45-52, wherein the medicament is for use with a steroid.