

WHAT IS CLAIMED IS:

1. An immunoconjugate comprising:
 - (a) a bispecific antibody that binds to EGFR and MUC1, the bispecific
5 antibody comprising:
 - (i) a first polypeptide comprising a first engineered Fc domain and a single-chain Fv (scFv), wherein the scFv binds MUC1,
 - (ii) a second polypeptide comprising a second engineered Fc domain and a heavy chain of a Fab fragment, and
 - 10 (iii) a third polypeptide comprising a light chain of the Fab fragment;
wherein the second and third polypeptide chains together define an Fab fragment that binds EGFR,
wherein the first polypeptide and the second polypeptide are covalently linked by one or more disulfide bonds formed between the first engineered Fc domain and
15 the second engineered Fc domain;
wherein the second polypeptide and the third polypeptide are covalently linked by one or more disulfide bonds formed between the heavy chain of the second polypeptide and the light chain of the third polypeptide; and
wherein the first polypeptide and the second polypeptides each comprise at
20 least one non-natural amino acid residue; and
 - (b) a plurality of hemiasterlin moieties, wherein each hemiasterlin moiety is independently conjugated via a linker to one of the non-natural amino acid residues of the first polypeptide or the second polypeptide.
2. The immunoconjugate of claim 1, wherein the plurality of hemiasterlin
25 moieties comprises four hemiasterlin moieties.
3. The immunoconjugate of claim 1 or 2, wherein the first engineered Fc domain is different from the second engineered Fc domain.
4. The immunoconjugate of claim 3, wherein the first and second engineered Fc domains each comprise strand-exchange engineered domains.

5. The immunoconjugate of claim 4, wherein each strand-exchange engineered domain comprises alternating segments of human IgA and IgG constant heavy chain-3 (CH3) sequences.
6. The immunoconjugate of any one of claims 1-5, wherein the first engineered Fc domain comprises two non-natural amino acid residues.
7. The immunoconjugate of claim 6, wherein the first engineered Fc domain comprises no more than two non-natural amino acid residues.
8. The immunoconjugate of claim 6 or 7, wherein the first engineered Fc domain comprises non-natural amino acid residues at heavy chain positions F241 and F404 according to the EU index.
9. The immunoconjugate of any one of claims 1-8, wherein the second engineered Fc domain comprises a non-natural amino acid residue.
10. The immunoconjugate of claim 9, wherein the second engineered Fc domain comprises no more than one non-natural amino acid residue.
11. The immunoconjugate of claim 9 or 10, wherein the second engineered Fc domain comprises a non-natural amino acid residue at heavy chain position F241 according to the EU index.
12. The immunoconjugate of any one of claims 1-11, wherein the Fab fragment comprises a non-natural amino acid residue.
13. The immunoconjugate of claim 12, wherein the heavy chain of the Fab fragment within the second polypeptide comprises a non-natural amino acid residue.
14. The immunoconjugate of claim 12 or 13, wherein the Fab fragment comprises no more than one non-natural amino acid residue.
15. The immunoconjugate of claim 12, 13, or 14, wherein the Fab fragment comprises a non-natural amino acid residue at heavy chain position Y180 according to the EU index.
16. The immunoconjugate of any one of claims 1-15, wherein each of the at least one non-natural amino acid residues is selected from the group consisting of p-acetyl-L-phenylalanine, O-methyl-L-tyrosine, 3-methyl-phenylalanine, O-4-allyl-L-tyrosine, 4-propyl-L-tyrosine, fluorinated phenylalanine, isopropyl-L-phenylalanine, p-azido-L-

phenylalanine, p-acyl-L-phenylalanine, p-benzoyl-L-phenylalanine, p-iodophenylalanine, p-bromophenylalanine, p-amino-L-phenylalanine, isopropyl-L-phenylalanine, p-propargyloxyphenylalanine, and p-azidomethyl-L-phenylalanine.

17. The immunoconjugate of claim 16, wherein each of the at least one non-natural amino acid residues is para-azidomethyl-L-phenylalanine (pAMF).

18. The immunoconjugate of any one of claims 1-17, wherein the bispecific antibody is aglycosylated.

19. The immunoconjugate of any one of claims 1-18, wherein the first polypeptide comprises complementarity-determining regions (CDRs):

10 CDR-L1 comprising the amino acid sequence set forth in SEQ ID NO:7;
CDR-L2 comprising the amino acid sequence set forth in SEQ ID NO:8; and
CDR-L3 comprising the amino acid sequence set forth in SEQ ID NO:9.

20. The immunoconjugate of claim 19, wherein the first polypeptide comprises complementarity-determining regions (CDRs):

15 CDR-H1 comprising the amino acid sequence set forth in SEQ ID NO:4,
CDR-H2 comprising the amino acid sequence set forth in SEQ ID NO:5, and
CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO:6.

21. The immunoconjugate of claim 19, wherein the first polypeptide comprises complementarity-determining regions (CDRs):

20 (a) (i) CDR-H1 comprising the amino acid sequence set forth in SEQ ID NO:29,
(ii) CDR-H2 comprising the amino acid sequence set forth in SEQ ID NO:30,
and
(iii) CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO:31;
or
25 (b) (i) CDR-H1 comprising the amino acid sequence set forth in SEQ ID NO:32,
(ii) CDR-H2 comprising the amino acid sequence set forth in SEQ ID NO:33,
and
(iii) CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO:34.

22. The immunoconjugate of claim 21, wherein the first polypeptide comprises:
- (a) a heavy chain variable (VH) region comprising the amino acid sequence set forth in SEQ ID NO:41; and
 - (b) a light chain variable (VL) region comprising the amino acid sequence set forth in SEQ ID NO:43.
23. The immunoconjugate of claim 19, wherein the first polypeptide comprises complementarity-determining regions (CDRs):
- (a) (i) CDR-H1 comprising the amino acid sequence set forth in SEQ ID NO:35,
(ii) CDR-H2 comprising the amino acid sequence set forth in SEQ ID NO:36,
and
(iii) CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO:37;
or
 - (b) (i) CDR-H1 comprising the amino acid sequence set forth in SEQ ID NO:38,
(ii) CDR-H2 comprising the amino acid sequence set forth in SEQ ID NO:39, and
(iii) CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO:40.
24. The immunoconjugate of claim 23, wherein the first polypeptide comprises:
- (a) a heavy chain variable (VH) region comprising the amino acid sequence set forth in SEQ ID NO:42; and
 - (b) a light chain variable (VL) region comprising the amino acid sequence set forth in SEQ ID NO:43.
25. The immunoconjugate of any one of claims 1-24, wherein the second polypeptide comprises complementarity-determining regions (CDRs):
- CDR-H1 comprising the amino acid sequence set forth in SEQ ID NO:13,
 - CDR-H2 comprising the amino acid sequence set forth in SEQ ID NO:14, and
 - CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO:15.
26. The immunoconjugate of any one of claims 1-25, wherein the third polypeptide comprises complementarity-determining regions (CDRs):

CDR-L1 comprising the amino acid sequence set forth in SEQ ID NO:16,

CDR-L2 comprising the amino acid sequence set forth in SEQ ID NO:17, and

CDR-L3 comprising the amino acid sequence set forth in SEQ ID NO:18.

5 27. The immunoconjugate of any one of claims 1-26, wherein the first polypeptide has an amino acid sequence at least 99% identical to that set forth in SEQ ID NO:1.

28. The immunoconjugate of claim 27, wherein the first polypeptide has an amino acid sequence as set forth in SEQ ID NO:11.

29. The immunoconjugate of any one of claims 1-28, wherein the second polypeptide has an amino acid sequence at least 99% identical to that set forth in SEQ ID NO:2.

30. The immunoconjugate of claim 29, wherein the second polypeptide has an amino acid sequence as set forth in SEQ ID NO:12.

31. The immunoconjugate of any one of claims 1-30, wherein the third polypeptide has an amino acid sequence at least 99% identical to that set forth in SEQ ID NO:3.

32. The immunoconjugate of claim 31, wherein the third polypeptide has an amino acid sequence as set forth in SEQ ID NO:3.

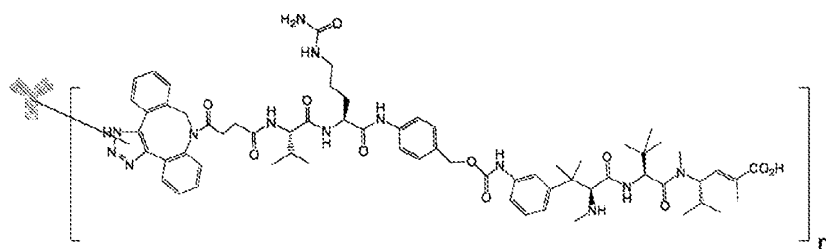
33. The immunoconjugate of any one of claims 1-32, wherein the linker is a cleavable linker.

20 34. The immunoconjugate of claim 33, wherein the cleavable linker is valine-citrulline-p-aminobenzylalcohol (PABA).

35. The immunoconjugate of any one of claims 1-34, wherein the hemiasterlin moiety is a hemiasterlin derivative.

25 36. The immunoconjugate of claim 35, wherein the hemiasterlin derivative is 3-aminophenyl-hemiasterlin.

37. The immunoconjugate of claim 36, wherein the immunoconjugate comprises the following structure:



wherein n is 4.

38. An immunoconjugate comprising:

(a) a bispecific antibody that binds to EGFR and MUC1, the bispecific antibody comprising:

(i) a first polypeptide comprising a first engineered Fc domain and a single-chain Fv fragment (scFv), wherein the scFv binds to MUC1, the first polypeptide chain comprising the amino acid sequence of SEQ ID NO:11 that comprises a non-natural amino acid residue at heavy chain positions F241 and F404 according to the EU index,

(ii) a second polypeptide comprising a second engineered Fc domain and a heavy chain of a Fab fragment, the second polypeptide comprising the amino acid sequence of SEQ ID NO:12 that comprises a non-natural amino acid residue at positions Y180 and F241 according to the EU index, and

(iii) a third polypeptide comprising a light chain of the Fab fragment, the third polypeptide comprising the amino acid sequence of SEQ ID NO:3;

wherein the second and third polypeptide chains together define a Fab fragment that binds EGFR,

wherein the first polypeptide and the second polypeptide are covalently linked by one or more disulfide bonds formed between the first engineered Fc domain and the second engineered Fc domain, and

wherein the second polypeptide and the third polypeptide are covalently linked by one or more disulfide bonds formed between the heavy chain of the second polypeptide and the light chain of the third polypeptide; and

(b) a plurality of 3-aminophenyl hemiasterlin moieties, each independently conjugated via a cleavable valine-citrulline-p-aminobenzylalcohol linker to one of the non-natural amino acid residues.

39. The immunoconjugate of claim 38, wherein the immunoconjugate comprises
5 four 3-aminophenyl hemiasterlin moieties.
40. The immunoconjugate of claim 39 or 40, wherein each non-natural amino acid is para-azidomethyl-L-phenylalanine (pAMF).
41. A pharmaceutical composition comprising the immunoconjugate of any one of claims 1-40 and a pharmaceutically acceptable carrier.
- 10 42. A method of treating cancer in a mammalian subject in need thereof, the method comprising the step of:
- administering a therapeutically effective amount of the immunoconjugate of any one of claims 1-40 or the pharmaceutical composition of claim 41 to the subject.
- 15 43. The method of claim 42, wherein the mammalian subject is a human.
44. The method of claim 42 or 43, wherein the mammalian subject is diagnosed as having cancer.
45. The method of any one of claims 42-44, wherein the cancer comprises a solid tumor.
- 20 46. The method of claim 45, wherein the cancer is selected from the group consisting of breast cancer, lung cancer, esophageal cancer, head and neck cancer, cervical cancer, ovarian cancer, gastric cancer, and mesothelioma.
47. The method of claim 46, wherein the cancer is breast cancer.
48. The method of claim 47, wherein the breast cancer is triple negative breast
25 cancer.
49. The method of claim 46, wherein the cancer is lung cancer.
50. The method of claim 49, wherein the lung cancer is a non-small cell lung cancer (NSCLC).
51. The method of claim 50, wherein the NSCLC comprises an adenocarcinoma.

52. The method of claim 50 or 51, wherein the NSCLC comprises a squamous cell carcinoma.
53. The method of claim 46, wherein the cancer is esophageal cancer.
54. The method of claim 53, wherein the esophageal cancer is squamous
5 esophageal cancer.
55. The method of claim 54, wherein the cancer is head and neck cancer.
56. The method of claim 55, wherein the head and neck cancer is head and neck squamous cell carcinoma.
57. The method of claim 46, wherein the cancer is cervical cancer.
- 10 58. The method of claim 46, wherein the cancer is ovarian cancer.
59. The method of claim 46, wherein the cancer is gastric cancer.
60. The method of claim 46, wherein the cancer is mesothelioma.
61. The method of any one of claims 42-44, wherein the cancer comprises a non-solid tumor.
- 15 62. The method of claim 61, wherein the cancer is multiple myeloma.
63. The method of any one of claims 42-62, wherein the cancer comprises cells that are wild type for EGFR.
64. The method of claim 63, wherein the cancer predominantly comprises cells that are wild type for EGFR.
- 20 65. The method of any one of claims 42-63, wherein the cancer comprises cells that comprise a mutant form of EGFR.
66. The method of claim 65, wherein the cancer predominantly comprises cells that comprise a mutant form of EGFR.
67. The method of any one of claims 42-66, wherein the cancer comprises cells
25 that express high levels of EGFR.
68. The method of claim 67, wherein the cancer predominantly comprises cells that express high levels of EGFR.

69. The method of any one of claims 42-67, wherein the cancer comprises cells that express low or moderate levels of EGFR.
70. The method of claim 69, wherein the cancer predominantly comprises cells that express low or moderate levels of EGFR.
- 5 71. The method of any one of claims 42-70, wherein the cancer comprises cells that express high levels of MUC1.
72. The method of claim 71, wherein the cancer predominantly comprises cells that express high levels of MUC1.
73. The method of any one of claims 42-71, wherein the cancer comprises cells
10 that express low or moderate levels of MUC1.
74. The method of claim 73, wherein the cancer predominantly comprises cells that express low or moderate levels of MUC1.
75. The method of any one of claims 42-74 wherein the step of administering comprises administration by a systemic route.
- 15 76. The method of claim 75, wherein the systemic route is an intravenous route.
77. The method of claim 75, wherein the systemic route is a subcutaneous route.
78. The method of any one of claims 42-77, wherein tumor growth is reduced relative to a reference level after administration of the immunoconjugate to the mammalian subject.
- 20 79. The method of claim 78, wherein the reference level is the level of tumor growth before the step of administering the immunoconjugate to the mammalian subject.
80. The method of claim 78 or 79, wherein tumor growth regresses after administration of the immunoconjugate to the mammalian subject.
- 25 81. The method of claim 80, wherein tumor growth regresses completely after administration of the immunoconjugate to the mammalian subject.
82. The method of any one of claims 42-81, wherein the step of administering comprises administering at least two doses, wherein the at least two doses collectively comprise a therapeutically effective amount.

83. The method of any one of claims 42-81, wherein the step of administering the immunoconjugate comprises administering a single dose that comprises a therapeutically effective amount.