

CLAIMS

1. 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 (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
 - (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 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 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 moieties comprises four hemiasterlin moieties.
3. The immunoconjugate of claim 1 or 2, wherein the first engineered Fc domain comprises two non-natural amino acid residues.

4. The immunoconjugate of claim 3, wherein the first engineered Fc domain comprises non-natural amino acid residues at heavy chain positions F241 and F404 according to the EU index.
5. The immunoconjugate of any one of claims 1-4, wherein the second engineered Fc domain comprises a non-natural amino acid residue.
6. The immunoconjugate of claim 5, wherein the second engineered Fc domain comprises a non-natural amino acid residue at heavy chain position F241 according to the EU index.
7. The immunoconjugate of any one of claims 1-6, wherein the Fab fragment comprises a non-natural amino acid residue.
8. The immunoconjugate of claim 7, wherein the Fab fragment comprises a non-natural amino acid residue at heavy chain position Y180 according to the EU index.
9. The immunoconjugate of any one of claims 1-8, 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.
10. The immunoconjugate of any one of claims 1-9, wherein the first polypeptide comprises complementarity-determining regions (CDRs):
 - 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.
11. The immunoconjugate of claim 10, wherein the first polypeptide comprises complementarity-determining regions (CDRs):
 - 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.

12. The immunoconjugate of claim 10, wherein the first polypeptide comprises complementarity-determining regions (CDRs):

- (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
- (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.

13. The immunoconjugate of claim 10, 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.

14. The immunoconjugate of any one of claims 1-13, 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.

15. The immunoconjugate of any one of claims 1-14, wherein the third polypeptide comprises complementarity-determining regions (CDRs):

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

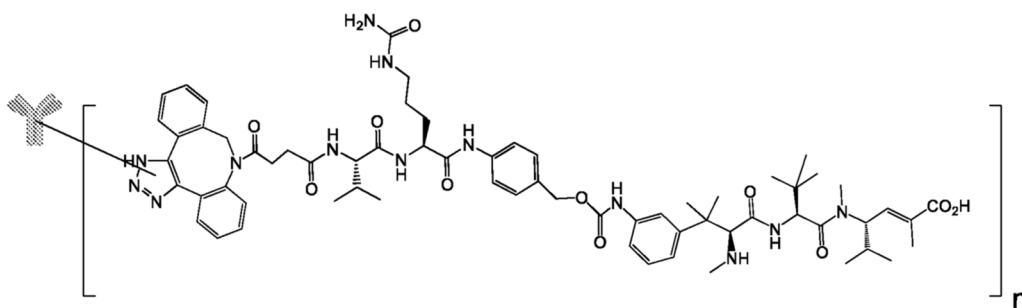
CDR-L2 comprising the amino acid sequence set forth in SEQ ID NO:17, and

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

16. The immunoconjugate of any one of claims 1-15, wherein the linker is a cleavable linker.

17. The immunoconjugate of any one of claims 1-16, wherein the hemiasterlin moiety is a hemiasterlin derivative.

18. The immunoconjugate of claim 17, wherein the immunoconjugate comprises the following structure:



wherein n is 4.

19. 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.

20. The immunoconjugate of claim 19, wherein the immunoconjugate comprises four 3-aminophenyl hemiasterlin moieties.

21. A pharmaceutical composition comprising the immunoconjugate of any one of claims 1-20 and a pharmaceutically acceptable carrier.

22. An immunoconjugate of any of claims 1-20 or a pharmaceutical composition thereof for use in treating cancer in a mammalian subject in need thereof.

23. Use of an immunoconjugate of any of claims 1-20 or a pharmaceutical composition thereof in the manufacture of a medicament for the treatment of cancer in a mammalian subject in need thereof.