

CLAIMS

1. A polypeptide complex comprising:

a first polypeptide comprising, from N-terminus to C-terminus, a first heavy chain variable domain (VH) of a first antibody operably linked to a first T cell receptor (TCR) constant region (C1), and

a second polypeptide comprising, from N-terminus to C-terminus, a first light chain variable domain (VL) of the first antibody operably linked to a second TCR constant region (C2),

wherein:

C1 and C2 are capable of forming a dimer comprising at least one non-native interchain bond between C1 and C2, and the non-native interchain bond is capable of stabilizing the dimer,

C1 comprises an engineered CBeta, and C2 comprises an engineered CAlpha,

the first VH is operably linked to C1 at a first junction domain, and the first VL is operably linked to C2 at a second junction domain, wherein the first junction domain comprises or is SEQ ID NO: 49 or 50, and the second junction domain comprises or is SEQ ID NO: 51 or 52,

and

the first antibody has a first antigenic specificity, wherein:

the polypeptide complex is not a bispecific polypeptide complex comprising a 1st antigen-binding moiety associated with a 2nd antigen-binding moiety, wherein:

the 1st antigen-binding moiety comprising:

a 1st polypeptide comprising, from N-terminus to C-terminus, a 1st heavy chain variable domain (VH) of a 1st antibody operably linked to a 1st T cell receptor (TCR) constant region (C1'), and

a 2nd polypeptide comprising, from N-terminus to C-terminus, a 1st light chain variable domain (VL) of the 1st antibody operably linked to a 2nd TCR constant region (C2'),

wherein:

C1' comprises an engineered CBeta comprising SEQ ID NO: 34 and C2' comprises an engineered CAlpha comprising SEQ ID NO: 44

the 2nd antigen-binding moiety comprising:

a 2nd VH of a 2nd antibody operably linked to an antibody heavy chain CH1 domain, and

a 2nd VL of the 2nd antibody operably linked to an antibody light chain constant (CL) domain,

wherein:

the 1st VH is operably linked to C1' at a 1st conjunction domain, and
the 1st VL is operably linked to C2' at a 2nd conjunction domain,
wherein the 1st conjunction domain comprises or is SEQ ID NO: 50, and
the 2nd conjunction domain comprises or is SEQ ID NO: 52,

wherein:

one of the 1st and the 2nd antigen-binding moiety is an anti-CD3 binding moiety, and the other one is an anti-CD19 binding moiety,

wherein: the anti-CD3 binding moiety is derived from an anti-CD3 antibody comprising:

a) a heavy chain CDR1 comprising SEQ ID NO: 342, b) a heavy chain CDR2 comprising SEQ ID NO: 343, c) a heavy chain CDR3 comprising SEQ ID NO: 344, d) a kappa light chain CDR1 comprising SEQ ID NO: 345, e) a kappa light

chain CDR2 comprising SEQ ID NO: 346, and f) a kappa light chain CDR3 comprising SEQ ID NO: 347; and

the anti-CD19 binding moiety is derived from an anti-CD19 antibody comprising:

a) a heavy chain CDR1 comprising SEQ ID NO: 356, b) a heavy chain CDR2 comprising SEQ ID NO: 359, c) a heavy chain CDR3 comprising SEQ ID NO: 358, d) a kappa light chain CDR1 comprising SEQ ID NO: 360, e) a kappa light chain CDR2 comprising SEQ ID NO: 361, and f) a kappa light chain CDR3 comprising SEQ ID NO: 362.

2. The polypeptide complex of claim 1, wherein the engineered CBeta and the engineered CAlpha comprise a pair of mutated cysteine residues that substitute for a pair of amino acid residues selected from the group consisting of: S56C in CBeta and T49C in CAlpha, S16C in CBeta and Y11C in CAlpha, F13C in CBeta and L13C in CAlpha, S16C in CBeta and L13C in CAlpha, V12C in CBeta and S16C in CAlpha, E14C in CBeta and S16C in CAlpha, F13C in CBeta and V23C in CAlpha, L62C in CBeta and Y44C in CAlpha, D58C in CBeta and T46C in CAlpha, S76C in CBeta and T46C in CAlpha, S56C in CBeta and L51C in CAlpha, S56C in CBeta and S62C in CAlpha, and R78C in CBeta and S62C in CAlpha, and wherein the pair of cysteine residues are capable of forming a non-native interchain disulphide bond, and wherein the native cysteine residue at position C74 of engineered CBeta is absent, and wherein the native CBeta comprises any one of SEQ ID NOs: 256 and 257, and the native CAlpha comprises SEQ ID NO: 254.

3. The polypeptide complex of claim 1, wherein at least one native glycosylation site is absent in the engineered CBeta and/or in the engineered CAlpha, wherein the native glycosylation site in the engineered CBeta is N69, and/or the native glycosylation

site(s) in the engineered CAlpha is/are selected from N34, N68, N79, and any combination thereof, wherein the native CBeta comprises any one of SEQ ID NOs: 256 and 257, and the native CAlpha comprises SEQ ID NO: 254.

4. The polypeptide complex of claim 1, wherein the engineered CBeta retains an FG loop encompassing the amino acid residues 101-117 of the native CBeta and/or a DE loop encompassing the amino acid residues 66-71 of the native CBeta,

wherein the native CBeta comprises any one of SEQ ID NOs: 256 and 257.

5. The polypeptide complex of claim 1, wherein the engineered CAlpha comprises SEQ ID NO: 43-48, and/or the engineered CBeta comprises SEQ ID NO: 33-41.

6. The polypeptide complex of claim 1, wherein the engineered CBeta lacks an FG loop encompassing the amino acid residues 101-117 of the native CBeta and/or a DE loop at position encompassing the amino acid residues 66-71 of the native CBeta,

wherein the native CBeta comprises any one of SEQ ID NOs: 256 and 257.

7. A conjugate comprising the polypeptide complex of claim 1, conjugated to a moiety.

8. An isolated polynucleotide encoding the polypeptide complex of claim 1, or an isolated vector comprising the polynucleotide.

9. A host cell comprising the isolated polynucleotide or the isolated vector of claim 8.

10. A method of producing the polypeptide complex of claim 1, comprising:

a) introducing to a host cell:

a first polynucleotide encoding a first polypeptide comprising, from N-terminus to C-terminus, a first heavy chain variable region (VH) of a first antibody operably linked to a first TCR constant region (C1), and
a second polynucleotide encoding a second polypeptide comprising, from N-terminus to C-terminus, a first light chain variable domain (VL) of the first antibody operably linked to a second TCR constant region (C2),
wherein:

C1 and C2 are capable of forming a dimer comprising at least one non-native interchain bond between C1 and C2, and the non-native interchain bond is capable of stabilizing the dimer,

C1 comprises an engineered CBeta, and C2 comprises an engineered CAlpha,

the first VH is operably linked to C1 at a first conjunction domain, and the first VL is operably linked to C2 at a second conjunction domain, wherein the first conjunction domain comprises or is SEQ ID NO: 49 or 50, and the second conjunction domain comprises or is SEQ ID NO: 51 or 52,
and

the first antibody has a first antigen-binding moiety, wherein:

the polypeptide complex is not a bispecific polypeptide complex comprising a 1st antigen-binding moiety associated with a 2nd antigen-binding moiety, wherein:

the 1st antigen-binding moiety comprising:

a 1st polypeptide comprising, from N-terminus to C-terminus, a 1st heavy chain variable domain (VH) of a 1st antibody operably linked to a 1st T cell receptor (TCR) constant region (C1'), and

a 2nd polypeptide comprising, from N-terminus to C-terminus, a 1st light chain variable domain (VL) of the 1st antibody operably linked to a 2nd TCR constant region (C2'),

wherein:

C1' comprises an engineered CBeta comprising SEQ ID NO: 34 and C2' comprises an engineered CAlpha comprising SEQ ID NO: 44,

the 2nd antigen-binding moiety comprising:

a 2nd VH of a 2nd antibody operably linked to an antibody heavy chain CH1 domain, and

a 2nd VL of the 2nd antibody operably linked to an antibody light chain constant (CL) domain,

wherein:

the 1st VH is operably linked to C1' at a 1st conjunction domain, and the 1st VL is operably linked to C2' at a 2nd conjunction domain, wherein the 1st conjunction domain comprises or is SEQ ID NO: 50, and the 2nd conjunction domain comprises or is SEQ ID NO: 52,

wherein:

one of the 1st and the 2nd antigen-binding moiety is an anti-CD3 binding moiety, and the other one is an anti-CD19 binding moiety,

wherein: the anti-CD3 binding moiety is derived from an anti-CD3 antibody comprising:

a) a heavy chain CDR1 comprising SEQ ID NO: 342, b) a heavy chain CDR2 comprising SEQ ID NO: 343, c) a heavy chain CDR3 comprising

SEQ ID NO: 344, d) a kappa light chain CDR1 comprising SEQ ID NO: 345, e) a kappa light chain CDR2 comprising SEQ ID NO: 346, and f) a kappa light chain CDR3 comprising SEQ ID NO: 347; and the anti-CD19 binding moiety is derived from an anti-CD19 antibody comprising:

a) a heavy chain CDR1 comprising SEQ ID NO: 356, b) a heavy chain CDR2 comprising SEQ ID NO: 359, c) a heavy chain CDR3 comprising SEQ ID NO: 358, d) a kappa light chain CDR1 comprising SEQ ID NO: 360, e) a kappa light chain CDR2 comprising SEQ ID NO: 361, and f) a kappa light chain CDR3 comprising SEQ ID NO: 362, and

b) allowing the host cell to express the polypeptide complex.

11. The polypeptide complex of claim 2, wherein the engineered CBeta and the engineered CAlpha comprise a pair of mutated cysteine residues: S56C in CBeta and T49C in CAlpha.

12. The polypeptide complex of claim 3, wherein the engineered CBeta and the engineered CAlpha comprise a pair of mutated cysteine residues: S56C in CBeta and T49C in CAlpha.

13. The polypeptide complex of claim 12, wherein the native glycosylation site N69 in the engineered CBeta is absent, and the native glycosylation sites N34, N68, and N79 in the engineered CAlpha are absent.

14. The polypeptide complex of claim 4, wherein the engineered CBeta and the engineered CAlpha comprise a pair of mutated cysteine residues: S56C in CBeta and T49C in CAlpha, wherein the native CAlpha comprises SEQ ID NO: 254.

15. The polypeptide complex of claim 14, wherein engineered CBeta retains an FG loop encompassing the amino acid residues 101-117 of the native CBeta and a DE loop encompassing the amino acid residues 66-71 of the native CBeta.

16. The polypeptide complex of claim 6, wherein the engineered CBeta and the engineered CAlpha comprise a pair of mutated cysteine residues: S56C in CBeta and T49C in Calpha, wherein the native CAlpha comprises SEQ ID NO: 254.

17. The polypeptide complex of claim 16, wherein the engineered CBeta lacks an FG loop encompassing the amino acid residues 101-117 of the native CBeta and a DE loop at position encompassing the amino acid residues 66-71 of the native CBeta.

18. The polypeptide complex of claim 1, wherein the engineered CAlpha comprises SEQ ID NO: 43, and the engineered CBeta comprises SEQ ID NO: 33.

19. The polypeptide complex of claim 1, wherein the engineered CAlpha comprises SEQ ID NO: 43, and the engineered CBeta comprises SEQ ID NO: 37.

20. The polypeptide complex of claim 1, wherein the engineered CAlpha comprises SEQ ID NO: 43, and the engineered CBeta comprises SEQ ID NO: 41.

21. The polypeptide complex of claim 1, wherein the engineered CAlpha comprises SEQ ID NO: 43, or the engineered CBeta comprises SEQ ID NO: 33.

22. The polypeptide complex of claim 1, wherein the engineered CAlpha comprises SEQ ID NO: 43, or the engineered CBeta comprises SEQ ID NO: 37.

23. The polypeptide complex of claim 1, wherein the engineered CAlpha comprises SEQ ID NO: 43, or the engineered CBeta comprises SEQ ID NO: 41.

24. The polypeptide complex of claim 11, wherein the first conjunction domain comprises or is SEQ ID NO: 50, and the second conjunction domain comprises or is SEQ

ID NO: 52.

25. The polypeptide complex of claim 13, wherein the first conjunction domain comprises or is SEQ ID NO: 50, and the second conjunction domain comprises or is SEQ ID NO: 52.

26. The polypeptide complex of claim 18, wherein the first conjunction domain comprises or is SEQ ID NO: 50, and the second conjunction domain comprises or is SEQ ID NO: 52.

27. The polypeptide complex of claim 1, wherein the engineered CAlpha comprises SEQ ID NO: 44, and the engineered CBeta comprises SEQ ID NO: 34.