

WHAT IS CLAIMED

1. A bispecific polypeptide complex, comprising a first antigen-binding moiety associated with a second antigen-binding moiety, wherein:

the first antigen-binding moiety comprises:

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 comprises an engineered CBeta comprising SEQ ID NO: 1 and C2 comprises an engineered CAlpha comprising SEQ ID NO: 2,

amino acid C48 in SEQ ID NO: 1 and amino acid C41 in SEQ ID NO: 2 are capable of forming a non-native interchain disulphide bond,

C1 and C2 are capable of forming a dimer, and the non-native interchain disulphide bond is capable of stabilizing the dimer, and

the second antigen-binding moiety comprises:

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

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

wherein:

one of the first and the second antigen-binding moiety is an anti-CD3 binding moiety, and the other one is an anti-CD19 binding moiety,

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

- a) a heavy chain CDR1 comprising SEQ ID NO: 3, b) a heavy chain CDR2 comprising SEQ ID NO: 4, c) a heavy chain CDR3 comprising SEQ ID NO: 5, d) a kappa light chain CDR1 comprising SEQ ID NO: 6, e) a kappa light chain

CDR2 comprising SEQ ID NO: 7, and f) a kappa light chain CDR3 comprising SEQ ID NO: 8;

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

a) a heavy chain CDR1 comprising SEQ ID NO: 9, b) a heavy chain CDR2 comprising SEQ ID NO: 10, c) a heavy chain CDR3 comprising SEQ ID NO: 11, d) a kappa light chain CDR1 comprising SEQ ID NO: 12, e) a kappa light chain CDR2 comprising SEQ ID NO: 13, and f) a kappa light chain CDR3 comprising SEQ ID NO: 14,

and

the first antigen-binding moiety and the second antigen-binding moiety are less prone to mispair than otherwise would have been if both the first and the second antigen-binding moieties are counterparts of natural Fab.

2. The bispecific polypeptide complex of claim 1, wherein the anti-CD3 binding moiety comprises a heavy chain variable domain sequence comprising SEQ ID NO: 15 and a light chain variable domain sequence comprising SEQ ID NO: 17.
3. The bispecific polypeptide complex of any of the preceding claims, wherein the anti-CD19 binding moiety comprises a heavy chain variable domain sequence comprising SEQ ID NO: 19 and a light chain variable domain sequence comprising SEQ ID NO: 21.
4. The bispecific polypeptide complex of any of the preceding claims, wherein:

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: 23, and/or the second conjunction domain comprises or is SEQ ID NO: 24.
5. The bispecific polypeptide complex of any of the preceding claims, wherein the first antigen-binding moiety is linked to a first dimerization domain, and the second antigen-binding moiety is linked to a second dimerization domain, wherein the first and the second dimerization domains are associated, and

wherein the association is via a connector, a disulphide bond, a hydrogen bond, electrostatic interaction, a salt bridge, or hydrophobic-hydrophilic interaction, or the combination thereof.
6. The bispecific polypeptide complex of claim 5, wherein the first and/or the second

dimerization domain comprises at least a portion of an antibody hinge region, optionally derived from IgG1, IgG2 or IgG4.

7. The bispecific polypeptide complex of claim 5, wherein the first and/or the second dimerization domain comprises an antibody CH2 domain, and/or an antibody CH3 domain.
8. The bispecific polypeptide complex of claim 5, wherein the first dimerization domain is operably linked to the first TCR constant region (C1) at a third conjunction domain, wherein the third conjunction domain comprises SEQ ID NO: 25.
9. The bispecific polypeptide complex of claim 5, wherein the second dimerization domain is operably linked to the heavy chain variable domain of the second antigen-binding moiety.
10. The bispecific polypeptide complex of claim 5, wherein the association is via knobs-into-holes.
11. The bispecific polypeptide complex of any of claims 1-10, wherein the bispecific polypeptide complex comprises a combination of four polypeptide sequences: SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, and SEQ ID NO: 29.
12. The bispecific polypeptide complex of any of claims 1-10, wherein the bispecific polypeptide complex comprises a combination of four polypeptide sequences: SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, and SEQ ID NO: 30.
13. A conjugate comprising the bispecific polypeptide complex of any of the preceding claims, conjugated to a moiety.
14. An isolated polynucleotide encoding the bispecific polypeptide complex of any of claims 1-12.
15. An isolated vector comprising the polynucleotide of claim 14.
16. A host cell comprising the isolated polynucleotide of claim 14 or the isolated vector of claim 15.
17. A method of producing a bispecific polypeptide complex, comprising:
 - a) introducing to a host cell one or more polynucleotides encoding the bispecific polypeptide complex of any of claims 1-12; and
 - b) allowing the host cell to express the bispecific polypeptide complex, and
 - c) isolating the bispecific polypeptide complex.
18. A pharmaceutical composition comprising the bispecific polypeptide complex of any of

claims 1-12 and a pharmaceutically acceptable carrier.

19. The bispecific polypeptide complex of any of claims 1-12 for use as a medicament.
20. The bispecific polypeptide complex of any of claims 1-12 for use in the treatment of a CD19-related disease or condition.
21. Use of the bispecific polypeptide complex of any of claims 1-12 in the manufacture of a medicament for the treatment of a CD19-related disease or condition,
wherein the disease or condition is cancer, wherein the cancer is lymphoma, lung cancer, liver cancer, cervical cancer, colon cancer, breast cancer, ovarian cancer, pancreatic cancer, melanoma, glioblastoma, prostate cancer, esophageal cancer or gastric cancer.
22. A kit comprising the bispecific polypeptide complex of any of claims 1-12, and instructions for using the bispecific polypeptide complex for detection, diagnosis, prognosis, or treatment of a CD19-related disease or condition.