

CLAIMS

1. A nucleic acid molecule encoding a chimeric CD3 fusion protein and a bispecific T cell activating element;

wherein the chimeric CD3 fusion protein comprises one or more polypeptide(s) (EC) recognizable by an anti-CD3 antibody, transmembrane domains (TM), co-stimulatory domains and CD3 signal activation domains; wherein EC is polypeptide from or derived from CD3e;

and wherein the bispecific T cell activating element is a fusion protein which comprises one or more tumor antigen recognition region(s) and one or more CD3 antigen-binding antibody fragment(s).

2. The nucleic acid molecule according to claim 1, wherein the chimeric CD3 fusion protein comprises one or more of the following domains: hinge or linker regions.

3. The nucleic acid molecule according to claim 1, wherein the fusion protein has a structure shown in the following formula I:



wherein in the formula,

L is absent or a signal peptide sequence;

EC is a polypeptide binding domain recognizable by an anti-CD3 antibody and binds to the anti-CD3 antibody;

H is absent or a linker or a hinge region;

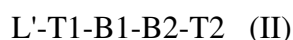
TM is a transmembrane domain;

C is absent or a costimulatory signaling molecule;

CD3 ζ is absent or a cytoplasmic signaling sequence derived from CD3 ζ ;

each "-" is independently a linker peptide or a peptide bond.

4. The nucleic acid molecule according to claim 1, wherein the bispecific T cell activating element has a structure shown in the following formula II: :



wherein:

L' is absent or a signal peptide sequence;

T1 is absent or a tag element;

B1 is a tumor antigen recognition region or a CD3 antigen-binding antibody fragment;

B2 is a CD3 antigen-binding antibody fragment or a tumor antigen recognition region; T2 is absent or a tag element;

each "-" is independently a linker peptide or peptide bond.

5. The nucleic acid molecule according to claim 1, wherein EC comprises position 1 to 104 of a CD3e protein, having an amino acid sequence as set forth by SEQ ID NO: 4.

6. The nucleic acid molecule according to claim 3, wherein C when present is a costimulatory signal molecule of a protein selected from the following group: OX40, CD2, CD7, CD27, CD28, CD30, CD40, CD70, CD134, 4-1BB (CD137), PD1, Dap10, CDS, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), NKG2D, GITR, TLR2, or a combination thereof.

7. The nucleic acid molecule according to claim 3, wherein C when present comprises a 4-1BB-derived costimulatory signal molecule having an amino acid sequence as set forth by SEQ ID NO: 10, and/or a CD28-derived costimulatory signal molecule having an amino acid sequence as set forth by SEQ ID NO: 62.

8. The nucleic acid molecule according to claim 3, wherein CD3 ζ when present is a cytoplasmic signaling sequence having an amino acid sequence as set forth by SEQ ID NO: 12.

9. The nucleic acid molecule according to claim 4, wherein B1 is a tumor antigen recognition region and B2 is a CD3 antigen-binding antibody fragment.

10. The nucleic acid molecule according to claim 4, wherein the tumor antigen recognition region comprises receptor or ligand binding domain, an antibody fragment, and/or a T-cell receptor (TCR) sequence.

11. The nucleic acid molecule according to claim 4, wherein the tumor antigen recognition region targets tumor antigen CAIX and/or HER2.

12. The nucleic acid molecule according to claim 4, wherein the tumor antigen recognition region is an antibody fragment, and having an amino acid sequence as set forth by SEQ ID NO: 18.

13. The nucleic acid molecule according to claim 4, wherein the tumor antigen recognition region is an antibody fragment, targeting HER2 and having an amino acid sequence as set forth by SEQ ID NO: 66.

14. The nucleic acid molecule according to claim 4, wherein the CD3 antigen- binding antibody fragment(s) comprises a single domain antibody sequence (VHH), a single-chain antibody variable region sequence (scFv), and/or an antigen-binding fragment (Fab) that targets CD3.

15. The nucleic acid molecule according to claim 4, wherein the CD3 antigen-binding antibody fragment is derived from anti-CD3 Ab clones.

16. The nucleic acid molecule according to claim 1, comprising the sequence as set forth by SEQ ID NO.: 73.

17. The nucleic acid molecule according to claim 1, comprising the sequence as set forth by SEQ ID. NO.: 67.

18. A vector, wherein the vector comprises the nucleic acid molecule according to any one of claims 1-17.

19. A genetically engineered immune cell, wherein the immune cell expresses the protein encoded by the nucleic acid molecule according to any one of claims 1-17.

20. The genetically engineered immune cell according to claim 19, wherein the immune cell is T cell.

21. A non-naturally occurring T cell population, comprising the genetically engineered immune cell according to claim 20, wherein the T cells are present in the T cell population at a ratio C1 of 10% or more, based on the total number of T cells in the T cell population.

22. A composition, comprising: (a) the genetically engineered immune cell according to claim 20 and/or the T cell population according to claim 21, and (b) a pharmaceutically acceptable carrier, diluent and/or excipient.

23. Use of the genetically engineered immune cell according to claim 20 and/or the T cell population according to claim 21, in the preparation of a medicament for the prevention and/or treatment of cancer or tumor.

24. The use according to claim 23, wherein the tumor is selected from the following group: a hematological tumor, a solid tumor, or a combination thereof.

25. The use according to claim 23, wherein the medicament is to be used in combination with dasatinib.