

## CLAIMS

1. A nucleic acid molecule encoding a chimeric CD3 fusion protein and a bispecific T cell activating element.
2. The nucleic acid molecule according to claim 1, wherein the chimeric CD3 fusion protein comprises of one or more polypeptide(s) recognizable by an anti-CD3 antibody, and optionally one or more of the following domains: hinge or linker regions, transmembrane domains (TM), co-stimulatory domains and CD3 signal activation domains.
3. The nucleic acid molecule according to claim 1, the bispecific T cell activating element is a fusion protein which comprises of one or more tumor antigen recognition region(s) and one or more CD3 antigen-binding antibody fragment(s).
4. 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;

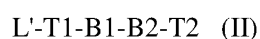
TM is a transmembrane domain;

C is absent or a costimulatory signaling molecule;

CD3 $\zeta$  is absent or a cytoplasmic signaling sequence derived from CD3 $\zeta$ ;

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

5. 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.

6. The nucleic acid molecule according to claim 4, wherein EC is a polypeptide from or derived from CD3e.

7. The nucleic acid molecule according to claim 6, wherein EC comprises position 1 to 104 of a CD3e protein, having a sequence as set forth by SEQ ID NO.: SEQ 3.

8. The nucleic acid molecule according to claim 4, 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.

9. The nucleic acid molecule according to claim 4, wherein C when present comprises a 4-1BB-derived costimulatory signal molecule having a sequence as set forth by SEQ ID NO.: 9, and/or a CD28-derived costimulatory signal molecule having a sequence as set forth by SEQ ID NO.: 61.

10. The nucleic acid molecule according to claim 4, wherein CD3ζ when present is a cytoplasmic signaling sequence as set forth by SEQ ID NO.: 11.

11. The nucleic acid molecule according to claim 1, wherein the construct is co-expressed with polypeptides from or derived from Caspase 9 (iCasp9), CD19, CD20, EGFR, HER2, CD30, CD19, c-Met, Claudin 18.2, or a combination thereof.

12. The nucleic acid molecule according to claim 5, wherein B1 is a tumor antigen recognition region and B2 is a CD3 antigen recognition region.

13. The nucleic acid molecule according to claim 5, wherein the tumor antigen recognition region comprises of receptor or ligand binding domain, an antibody fragment, and/or a T-cell receptor (TCR) sequence.

14. The nucleic acid molecule according to claim 5, wherein the tumor antigen recognition region targets tumor antigen CAIX and/or HER2.

15. The nucleic acid molecule according to claim 5, wherein the tumor antigen recognition region is an antibody fragment, and having a sequence as set forth by SEQ ID NO.: 17.

16. The nucleic acid molecule according to claim 5, wherein the tumor antigen recognition region is an antibody fragment, targeting HER2 and having a sequence as set forth by SEQ ID NO.: 65.

17. The nucleic acid molecule according to claim 5, wherein the CD3 antigen-binding antibody fragment(s) comprises of a single domain antibody sequence (VHH), a single-chain antibody variable region sequence (scFv), and/or an antigen-binding fragment (Fab) that targets CD3.

18. The nucleic acid molecule according to claim 5, wherein the CD3 antigen-binding antibody fragment is derived from anti-CD3 Ab clones of L2K, UCHT, OKT3, F6A, SP34 etc.

19. The nucleic acid molecule according to any one of the preceding claims, comprising the sequence as set forth by SEQ ID NO.: 73.

20. The nucleic acid molecule according to any one of the preceding claims, comprising the sequence as set forth by SEQ ID. NO.: 67.

21. The nucleic acid molecule according to claim 1, wherein the nucleic acid molecule encoding the chimeric CD3 fusion protein and the nucleic acid molecule encoding the bispecific T cell activating element are encoded by separate nucleic acid molecules.

22. The nucleic acid molecule according to claim 21, wherein the nucleic acid molecule encoding the chimeric CD3 fusion protein and the nucleic acid molecule encoding the bispecific T cell activating element are co-expressed in the same immune cell.

23. A vector, wherein the vector comprises the nucleic acid molecule according to any one of the preceding claims.

24. A genetically engineered immune cell, wherein the immune cell expresses the nucleic acid molecule according to any one of the preceding claims.

25. The genetically engineered immune cell according to claim 24, wherein the immune cell is T cell.

26. The genetically engineered immune cell according to claim 25, wherein the immune cell is engineered to express the chimeric CD3 fusion protein and the bispecific T cell activating element, whereby the nucleic acid molecule encoding chimeric CD3 fusion protein and the nucleic acid molecule encoding the bispecific T cell activating element are not provided on the same DNA construct.

27. A non-naturally occurring T cell population, wherein the T cells according to claim 25 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.

28. A composition, comprising: (a) the T cell according to claim 25 and/or the T cell population according to claim 27, and (b) a pharmaceutically acceptable carrier, diluent and/or excipient.

29. A method of preventing and/or treating cancer or tumor, comprising administering the genetically engineered T cell according to claim 25 and/or the T cell population according to claim 27 to a subject in need thereof.

30. The method according to claim 29, wherein the tumor is selected from the following group: a hematological tumor, a solid tumor, or a combination thereof.

31. The method according to claim 29, wherein the method further comprises the administration of dasatinib.

32. Use of the genetically engineered T cell according to claim 25 and/or the T cell population according to claim 27, in the preparation of a medicament for the prevention and/or treatment of cancer or tumor.

33. The use according to claim 32, wherein the tumor is selected from the following group: a hematological tumor, a solid tumor, or a combination thereof.

34. The use according to claim 32, wherein the medicament is used in combination with dasatinib.

35. A method of reducing the toxicity of an immune cell engineered with a chimeric CD3 fusion protein and a bispecific T cell activating element, comprising the administration of dasatinib.

36. Use of dasatinib in the preparation of a medicament for reducing the toxicity of an immune cell engineered with a chimeric CD3 fusion protein and a bispecific T cell activating element.