

CLAIMS

1. A ~~multimeric-trimeric~~ protein comprising ~~at least~~ three monomer polypeptides, wherein each monomer polypeptide comprises (1) a first 4-1BB-targeting ~~moiety-lipocalin mutein~~ (T1), and (2) an ~~oligomerization-trimerization~~ moiety (O).
2. The ~~multimeric-trimeric~~ protein of claim 1, wherein the first 4-1BB-targeting ~~moiety lipocalin mutein~~ (T1) is fused at its N-terminus or C-terminus to the C-terminus or N-terminus, respectively, of the oligomerization moiety (O) via a linker (L).
3. The ~~multimeric-trimeric~~ protein of claim 1 or 2, wherein the monomer polypeptide ~~further~~ comprises ~~at least one~~ (3) an additional targeting moiety (T2).
4. The ~~multimeric-trimeric~~ protein of any one of claims 1-3, wherein the monomer polypeptide ~~further~~ comprises (3) an additional targeting moiety (T2), wherein the additional targeting moiety (T2) is placed in tandem with the first 4-1BB-targeting moiety (T1);
- ~~5. The multimeric protein of claim 4,~~ wherein the monomer polypeptide has preferably one of the following configurations:
 - a. T1-L'-T2-L-O;
 - b. T2-L'-T1-L-O;
 - c. O-L-T1-L'-T2; or
 - d. O-L-T2-L'-T1;wherein L' is a linker that is the same as or different from L.
- ~~6~~5. The ~~multimeric-trimeric~~ protein of any one of claims 1-3, wherein the monomer polypeptide ~~further~~ comprises (3) an additional targeting moiety (T2), wherein the additional targeting moiety (T2) is linked to a different terminus of the oligomerization moiety (O) than the first 4-1BB-targeting moiety (T1);
- ~~7. The multimeric protein of claim 6,~~ wherein the monomer polypeptide preferably has one of the following configurations:
 - a. T1-L-O-L'-T2; or

b. T2-L'-O-L-T1;

wherein L' is a linker that is the same as or different from L.

~~86.~~ The ~~multimeric~~-trimeric protein of any one of claims 3-~~75~~, wherein the additional targeting moiety (T2) is a second 4-1BB-targeting ~~moiety~~lipocalin mutein, wherein the second 4-1BB-targeting lipocalin mutein is preferably the same as the first 4-1BB-targeting lipocalin mutein.

~~9.~~ The multimeric protein of claim 8, wherein the second 4-1BB-targeting moiety is the same as the first 4-1BB-targeting moiety (T1).

~~10.~~ The multimeric protein of any one of claims 3-7, wherein the additional targeting moiety (T2) is a moiety that targets a tumor associated antigen.

~~11.~~ The multimeric protein of any one of claims 3-7, wherein the additional targeting moiety (T2) is a moiety that targets a tumor associated antigen and is a lipocalin mutein.

~~12.~~ The multimeric protein of any one of claims 3-7, wherein the additional targeting moiety (T2) is a moiety that targets a tumor associated antigen and is an antibody or an antigen binding domain or derivative thereof.

~~13.~~ The multimeric protein of any one of claims 3-7, wherein the additional targeting moiety (T2) is a moiety that targets a tumor associated antigen and is a single chain variable fragment (scFv).

~~14.~~ The multimeric protein of any one of claims 3-7, wherein the additional targeting moiety (T2) is a GPC3-targeting moiety.

~~15.~~ The multimeric protein of any one of claims 3-7, wherein the additional targeting moiety (T2) is a GPC3-targeting moiety that is a lipocalin mutein.

~~16.~~ The multimeric protein of claim 15, wherein the lipocalin mutein has at least 85% sequence identity to an amino acid sequence selected from the group consisting of SEQ

~~ID NOs: 74-97.~~

- ~~17. The multimeric protein of any one of claims 3-7, wherein the additional targeting moiety (T2) is a GPC3 targeting moiety that is an antibody or an antigen binding domain or derivative thereof.~~
- ~~18. The multimeric protein of any one of claims 3-7, wherein the additional targeting moiety (T2) is a GPC3 targeting moiety that is a single chain variable fragment (scFv).~~
- ~~19. The multimeric protein of any one of claims 3-7, wherein the additional targeting moiety (T2) is a PD-L1 targeting moiety.~~
- ~~20. The multimeric protein of any one of claims 3-7, wherein the additional targeting moiety (T2) is a PD-L1 targeting moiety that is a single chain variable fragment (scFv).~~
- ~~21. The multimeric protein of claim 20, wherein the scFv has at least 85% sequence identity to the amino acid sequence shown in SEQ ID NO: 172.~~
227. The ~~multimeric~~ trimeric protein of any one of claims 3-~~7~~5, wherein the additional targeting moiety (T2) is a T cell activation enhancing targeting moiety.
- ~~23. The multimeric protein of any one of claims 3-7, wherein the additional targeting moiety (T2) is a T cell activation enhancing targeting moiety that is a lipocalin mutein.~~
- ~~24. The multimeric protein of any one of claims 3-7, wherein the additional targeting moiety (T2) is a T cell activation enhancing targeting moiety that is an antibody or an antigen binding domain or derivative thereof.~~
- ~~25. The multimeric protein of any one of claims 3-7, wherein the additional targeting moiety (T2) is a T cell activation enhancing targeting moiety that is a single chain variable fragment (scFv).~~
- ~~26. The multimeric protein of any one of claims 3-7, wherein the additional targeting moiety (T2) is an OX40 targeting moiety.~~

~~278.~~ The ~~multimeric-trimeric~~ protein of any one of claims 3-~~75~~, wherein the additional targeting moiety (T2) is an OX40-targeting ~~moiety that is a~~ lipocalin mutein.

~~289.~~ The ~~multimeric-trimeric~~ protein of claim ~~278~~, wherein the lipocalin mutein has at least 85% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 174-202.

~~29.~~ The ~~multimeric~~ protein of any one of claims 1-28, wherein the first 4-1BB-targeting moiety (T1) is a lipocalin mutein.

~~3010.~~ The ~~multimeric-trimeric~~ protein of any one of claims 1-~~299~~, wherein the first 4-1BB-targeting ~~moiety-lipocalin mutein~~ (T1) is a lipocalin mutein having at least 85% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 56-71.

~~31.~~ The ~~multimeric~~ protein of any one of claims 1-30, wherein the oligomerization moiety (O) is capable of promoting trimerization.

~~3211.~~ The ~~multimeric-trimeric~~ protein of any one of claims 1-~~3410~~, wherein the ~~oligomerization-trimerization~~ moiety (O) is a trimerization domain of a collagen.

~~3312.~~ The ~~multimeric-trimerization~~ protein of any one of claims 1-~~3211~~, wherein the oligomerization moiety (O) comprises the amino acid sequence of SEQ ID NO: 35 or has at least 85% sequence identity to an-the amino acid sequence ~~selected from the group consisting of~~ SEQ ID NOs: 35-~~37~~.

~~34.~~ The ~~multimeric~~ protein of any one of claims 1-33, wherein the ~~multimeric~~ protein is a trimeric protein.

~~35.~~ The ~~multimeric~~ protein of any one of claims 1-30 and 33, wherein the ~~multimeric~~ protein is a tetrameric protein.

~~3613.~~ The ~~multimeric-trimeric~~ protein of any one of claims 2-~~3512~~, wherein the linker (L) has

at least 85% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 12-28.

~~37~~14. The ~~multimeric-trimeric~~ protein of any one of claims 1-~~36~~13, wherein the ~~multimeric trimeric~~ protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 38-45, 48-53 and 164-166 or comprises an amino acid sequence that has at least 85% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 38-~~45, 48-53~~55 and 164-~~166~~167.

~~38. The multimeric protein of any one of claims 1-37, wherein the multimeric protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 38-55 and 164-167.~~

~~39. The multimeric protein of any one of claims 1-38, wherein the multimeric protein is capable of binding 4-1BB with a K_D value of about 0.68 nM or lower.~~

~~40. The multimeric protein of any one of claims 1-39, wherein the multimeric protein is capable of binding 4-1BB with an apparent K_D value lower than the K_D value of the 4-1BB targeting lipocalin mutein that is included in the monomer polypeptide.~~

~~41. The multimeric protein of claim 39 or 40, wherein the apparent K_D value is determined by surface plasmon resonance (SPR).~~

~~42. The multimeric protein of any one of claims 1-41, wherein the multimeric protein is cross-reactive with cynomolgus 4-1BB.~~

~~43~~15. A nucleic acid molecule comprising a nucleotide sequence encoding a monomer polypeptide comprised in a ~~multimeric-trimeric~~ protein of any one of claims 1-~~42~~14.

~~44. The nucleic acid molecule of claim 43, wherein the nucleic acid molecule is operably linked to a regulatory sequence to allow expression of said nucleic acid molecule.~~

~~45. The nucleic acid molecule of claim 43 or 44, wherein the nucleic acid molecule is comprised in a vector or in a phagemid vector.~~

~~46. The nucleic acid molecule of any one of claims 43 or 44, wherein the nucleic acid molecule is comprised in a viral vector, in a nanoparticle, or a liposome.~~

~~47. The nucleic acid molecule of any one of claims 43-46, wherein the nucleic acid molecule is comprised in the genomic DNA of a host cell.~~

~~48~~16. A cell containing a nucleic acid molecule of ~~any one of claims 43-47~~15 and/or expressing the ~~multimeric-trimeric~~ protein of any one of claims 1-~~42-14~~ and/or expressing a monomer polypeptide as defined in any one of claims 1-~~42~~14.

~~49. The cell of claim 48, wherein the cell secretes the multimeric protein and/or the monomer polypeptide.~~

~~50. The cell of claim 48, wherein the cell secretes the monomer polypeptide.~~

~~51. The cell of claim 50, wherein the monomer polypeptide self-assembles to a multimeric protein after secretion.~~

~~52~~17. The cell of ~~any one of claims 48-51~~16, wherein the cell is an immune cell, preferably a CAR-T cell.

~~53. The cell of claim 52, wherein the cell is a T cell.~~

~~54. The cell of claim 53, wherein the cell is a CD8+ T cell.~~

~~55. The cell of claim 53, wherein the cell is a CD4+ T cell.~~

~~56. The cell of any one of claims 52-55, wherein the cell comprises a recombinant antigen receptor.~~

~~57. The cell of claim 56, wherein the recombinant antigen receptor is a chimeric antigen receptor (CAR).~~

- ~~58. The cell of claim 56, wherein the recombinant antigen receptor is a T cell receptor (TCR).~~
- ~~59. The cell of any one of claims 52-57, wherein the cell is a CAR-T cell.~~
- ~~60. The cell of any one of claims 52-59, wherein the cell expresses 4-1BB.~~
- ~~61. The cell of any one of claims 52-60, wherein the cell is a human cell.~~
- ~~62. A method of producing the multimeric protein of any one of claims 1-42, wherein the multimeric protein is produced starting from the nucleic acid coding for the monomer polypeptides comprised in the multimeric protein.~~
- ~~63. The method of claim 62, wherein the multimeric protein is produced in a bacterial or eukaryotic host organism.~~
- ~~64. A use of the multimeric protein of any one of claims 1-42 or a composition comprising such multimeric protein or a cell of any one of claims 48-61 for inducing 4-1BB (and/or, optionally, OX40) clustering and activation on T cells.~~
- ~~65. A use of the multimeric protein of any one of claims 1-42 or a composition comprising such multimeric protein or a cell of any one of claims 48-61 for co-stimulating T cells and/or activating downstream signaling pathways of 4-1BB (and/or, optionally, OX40).~~
- ~~66. A use of the multimeric protein of any one of claims 1-42 or a composition comprising such multimeric protein or a cell of any one of claims 48-61 for co-stimulating T cells when engaging GPC3-expressing or PD-L1-expressing tumor cells.~~
- ~~67. The use of any one of claims 64-66, wherein the T cell is a T cell expressing the multimeric protein and/or one of its monomer polypeptides.~~
- ~~68. The use of any one of claims 64-66, wherein the T cell is a T cell not expressing the multimeric protein and/or one of its monomer polypeptides.~~

~~69~~18. A pharmaceutical composition comprising one or more ~~multimeric~~trimeric proteins of any one of claims 1-~~42~~14 and/or one or more cells of ~~any one of~~ claims ~~48-61~~16 or 17.

~~70~~19. The ~~multimeric~~trimeric protein of any one of claims 1-~~42~~14 and/or the cell of ~~any one of~~ claims ~~48-61~~16 or 17 for use in a therapy.

~~71.~~ The multimeric protein and/or the cell for use of claim ~~70~~, wherein the use is in the treatment of cancer.

~~72~~20. Use of a multimeric protein of any one of claims 1-~~42~~14 and/or the cell of ~~any one of~~ claims ~~48-61~~16 or 17 ~~for in~~ the manufacture of a medicament.

~~73.~~ The use of claim ~~72~~, wherein the medicament is preferably for the treatment of cancer.