

CLAIMS

1. An antibody cytokine engrafted protein comprising:
 - (a) a heavy chain variable region (VH), comprising Complementarity Determining Regions (CDRs) HCDR1, HCDR2, HCDR3; and
 - (b) a light chain variable region (VL), comprising LCDR1, LCDR2, LCDR3; and
 - (c) a mutated Interleukin 2 (IL2) molecule engrafted into the HCDR1,

wherein the HCDR1 engrafted with the mutated IL2 molecule comprises SEQ ID NO: 7, SEQ ID NO: 10, SEQ ID NO: 13, SEQ ID NO: 16, SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 45, or SEQ ID NO: 48, the HCDR2 comprises an amino acid sequence of SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 14, SEQ ID NO: 17, SEQ ID NO: 40, SEQ ID NO: 43, SEQ ID NO: 46 or SEQ ID NO: 49, the HCDR3 comprises an amino acid sequence of SEQ ID NO: 9, SEQ ID NO: 12, SEQ ID NO: 15, SEQ ID NO: 18, SEQ ID NO: 41, SEQ ID NO: 44, SEQ ID NO: 47 or SEQ ID NO: 50, the LCDR1 comprises an amino acid sequence of SEQ ID NO: 23, SEQ ID NO: 26, SEQ ID NO: 29, SEQ ID NO: 32, SEQ ID NO: 55, SEQ ID NO: 58, SEQ ID NO: 61 or SEQ ID NO: 64, the LCDR2 comprises an amino acid sequence of SEQ ID NO: 24, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NO: 33, SEQ ID NO: 56, SEQ ID NO: 59, SEQ ID NO: 62 or SEQ ID NO: 65, and the LCDR3 comprises an amino acid sequence of SEQ ID NO: 25, SEQ ID NO: 28, SEQ ID NO: 31, SEQ ID NO: 34, SEQ ID NO: 57, SEQ ID NO: 60, SEQ ID NO: 63 or SEQ ID NO: 66, and

wherein the mutated IL2 molecule contains a mutation that reduces the affinity of the mutated IL2 molecule to a high affinity IL2 receptor.
2. An antibody cytokine engrafted protein comprising:
 - (a) a heavy chain variable region (VH), comprising Complementarity Determining Regions (CDRs) HCDR1, HCDR2, HCDR3; and
 - (b) a light chain variable region (VL), comprising LCDR1, LCDR2, LCDR3; and
 - (c) a mutated Interleukin 2 (IL2) molecule engrafted into the HCDR1,

wherein the HCDR1 engrafted with the mutated IL2 molecule comprises SEQ ID NO: 7, SEQ ID NO: 10, SEQ ID NO: 13, SEQ ID NO: 16, SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 45, or SEQ ID NO: 48, the HCDR2 comprises an amino acid sequence of SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 14, SEQ ID NO: 17, SEQ ID NO: 40, SEQ ID NO: 43, SEQ ID NO: 46 or SEQ ID NO: 49, the HCDR3 comprises an amino acid

sequence of SEQ ID NO: 9, SEQ ID NO: 12, SEQ ID NO: 15, SEQ ID NO: 18, SEQ ID NO: 41, SEQ ID NO: 44, SEQ ID NO: 47 or SEQ ID NO: 50, the LCDR1 comprises an amino acid sequence of SEQ ID NO: 23, SEQ ID NO: 26, SEQ ID NO: 29, SEQ ID NO: 32, SEQ ID NO: 55, SEQ ID NO: 58, SEQ ID NO: 61 or SEQ ID NO: 64, the LCDR2 comprises an amino acid sequence of SEQ ID NO: 24, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NO: 33, SEQ ID NO: 56, SEQ ID NO: 59, SEQ ID NO: 62 or SEQ ID NO: 65, and the LCDR3 comprises an amino acid sequence of SEQ ID NO: 25, SEQ ID NO: 28, SEQ ID NO: 31, SEQ ID NO: 34, SEQ ID NO: 57, SEQ ID NO: 60, SEQ ID NO: 63 or SEQ ID NO: 66, and

wherein the antibody cytokine engrafted protein preferentially expands T effector cells over regulatory T cells.

3. An antibody cytokine engrafted protein comprising:

- (a) a heavy chain variable region (VH), comprising Complementarity Determining Regions (CDRs) HCDR1, HCDR2, HCDR3; and
- (b) a light chain variable region (VL), comprising LCDR1, LCDR2, LCDR3;
- (c) a mutated Interleukin 2 (IL2) molecule engrafted into the HCDR1,

wherein the HCDR1 engrafted with the mutated IL2 molecule comprises SEQ ID NO: 7, SEQ ID NO: 10, SEQ ID NO: 13, SEQ ID NO: 16, SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 45, or SEQ ID NO: 48, the HCDR2 comprises an amino acid sequence of SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 14, SEQ ID NO: 17, SEQ ID NO: 40, SEQ ID NO: 43, SEQ ID NO: 46 or SEQ ID NO: 49, the HCDR3 comprises an amino acid sequence of SEQ ID NO: 9, SEQ ID NO: 12, SEQ ID NO: 15, SEQ ID NO: 18, SEQ ID NO: 41, SEQ ID NO: 44, SEQ ID NO: 47 or SEQ ID NO: 50, the LCDR1 comprises an amino acid sequence of SEQ ID NO: 23, SEQ ID NO: 26, SEQ ID NO: 29, SEQ ID NO: 32, SEQ ID NO: 55, SEQ ID NO: 58, SEQ ID NO: 61 or SEQ ID NO: 64, the LCDR2 comprises an amino acid sequence of SEQ ID NO: 24, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NO: 33, SEQ ID NO: 56, SEQ ID NO: 59, SEQ ID NO: 62 or SEQ ID NO: 65, and the LCDR3 comprises an amino acid sequence of SEQ ID NO: 25, SEQ ID NO: 28, SEQ ID NO: 31, SEQ ID NO: 34, SEQ ID NO: 57, SEQ ID NO: 60, SEQ ID NO: 63 or SEQ ID NO: 66, and

wherein the antibody cytokine engrafted protein stimulates one or more of CD8 T effector cell, NK cell proliferation, or combination thereof, greater than recombinant IL2 or aldesleukin.

4. The antibody cytokine engrafted protein of any one of claims 1 to 3, wherein the mutated IL2 molecule comprises SEQ ID NO: 4.
5. The antibody cytokine engrafted protein of any one of claims 1 to 3, wherein the mutated IL2 molecule comprises SEQ ID NO: 6.
6. The antibody cytokine engrafted protein of any one of claims 1 to 3, wherein the VH comprises SEQ ID NO: 19 or SEQ ID NO: 51 or the VL comprises SEQ ID NO: 35 or SEQ ID NO: 67.
7. The antibody cytokine engrafted protein of any one of claims 1 to 6, further comprising an IgG class antibody heavy chain.
8. The antibody cytokine engrafted protein of claim 7, wherein the IgG class heavy chain is selected from IgG1, IgG2, and IgG4.
9. The antibody cytokine engrafted protein of claim 7, wherein the IgG class heavy chain comprises SEQ ID NO: 21 or SEQ ID NO: 53.
10. The antibody cytokine engrafted protein of any one of claims 1 to 3, wherein the IgG class light chain comprises SEQ ID NO:69 and the IgG class heavy chain comprises SEQ ID NO:53.
11. The antibody cytokine engrafted protein of any one of claims 1 to 3, wherein the IgG class light chain comprises SEQ ID NO:37 and the IgG class heavy chain comprises SEQ ID NO:21.
12. The antibody cytokine engrafted protein of any one of claims 1 to 3, wherein the IgG class light chain comprises SEQ ID NO:69 and the IgG class heavy chain comprises SEQ ID NO:21.

13. The antibody cytokine engrafted protein of any one of claims 1 to 3, wherein the IgG class light chain comprises SEQ ID NO:37 and the IgG class heavy chain comprises SEQ ID NO:53.
14. The antibody cytokine engrafted protein of any one of claims 1 to 3, comprising:
- (a) an IgG class heavy chain comprising an IgG class heavy chain variable region (VH), comprising Complementarity Determining Regions (CDR) HCDR1 engrafted with a mutated Interleukin 2 (IL2) molecule comprising SEQ ID NO: 7, HCDR2 comprising SEQ ID NO: 8, and HCDR3 comprising SEQ ID NO: 9; and
an IgG class light chain comprising an IgG class light chain variable region (VL), comprising LCDR1 comprising SEQ ID NO: 23, LCDR2 comprising SEQ ID NO: 24, and LCDR3 comprising SEQ ID NO: 25;
 - (b) an IgG class heavy chain comprising an IgG class VH, comprising HCDR1 engrafted with a mutated IL2 molecule comprising SEQ ID NO: 10, HCDR2 comprising SEQ ID NO: 11, and HCDR3 comprising SEQ ID NO: 12; and
an IgG class light chain comprising an IgG class VL, comprising LCDR1 comprising SEQ ID NO: 26, LCDR2 comprising SEQ ID NO: 27, and LCDR3 comprising SEQ ID NO: 28;
 - (c) an IgG class heavy chain comprising an IgG class VH, comprising HCDR1 engrafted with a mutated IL2 molecule comprising SEQ ID NO: 13, HCDR2 comprising SEQ ID NO: 14, and HCDR3 comprising SEQ ID NO: 15; and
an IgG class light chain comprising an IgG class VL, comprising LCDR1 comprising SEQ ID NO: 29, LCDR2 comprising SEQ ID NO: 30, and LCDR3 comprising SEQ ID NO: 31;
 - (d) an IgG class heavy chain comprising an IgG class VH, comprising HCDR1 engrafted with a mutated IL2 molecule comprising SEQ ID NO: 16, HCDR2 comprising SEQ ID NO: 17, and HCDR3 comprising SEQ ID NO: 18; and
an IgG class light chain comprising an IgG class VL, comprising LCDR1 comprising SEQ ID NO: 32, LCDR2 comprising SEQ ID NO: 33, and LCDR3 comprising SEQ ID NO: 34;
 - (e) an IgG class heavy chain comprising an IgG class VH, comprising HCDR1 engrafted with a mutated IL2 molecule comprising SEQ ID NO: 39, HCDR2 comprising SEQ ID NO: 40, and HCDR3 comprising SEQ ID NO: 41; and
an IgG class light chain comprising an IgG class VL, comprising LCDR1 comprising SEQ ID NO: 55, LCDR2 comprising SEQ ID NO: 56, and LCDR3 comprising SEQ ID NO: 57;
 - (f) an IgG class heavy chain comprising an IgG class VH, comprising HCDR1 engrafted with a mutated IL2 molecule comprising SEQ ID NO: 42, HCDR2 comprising SEQ ID NO:

43, and HCDR3 comprising SEQ ID NO: 44; and

an IgG class light chain comprising an IgG class VL, comprising LCDR1 comprising SEQ ID NO: 58, LCDR2 comprising SEQ ID NO: 59, and LCDR3 comprising SEQ ID NO: 60;

(g) an IgG class heavy chain comprising an IgG class VH, comprising HCDR1 engrafted with a mutated IL2 molecule comprising SEQ ID NO: 45, HCDR2 comprising SEQ ID NO: 46, and HCDR3 comprising SEQ ID NO: 47; and

an IgG class light chain comprising an IgG class VL, comprising LCDR1 comprising SEQ ID NO: 61, LCDR2 comprising SEQ ID NO: 62, and LCDR3 comprising SEQ ID NO: 63;

or

(h) an IgG class heavy chain comprising an IgG class VH, comprising HCDR1 engrafted with a mutated IL2 molecule comprising SEQ ID NO: 48, HCDR2 comprising SEQ ID NO: 49, and HCDR3 comprising SEQ ID NO: 50; and

an IgG class light chain comprising an IgG class VL, comprising LCDR1 comprising SEQ ID NO: 64, LCDR2 comprising SEQ ID NO: 65, and LCDR3 comprising SEQ ID NO: 66.

15. The antibody cytokine engrafted protein of any one of claims 1 to 3, wherein the IgG class light chain comprises 95% or more sequence identity with SEQ ID NO:37 and the IgG class heavy chain comprises 95% or more sequence identity with SEQ ID NO:21.

16. The antibody cytokine engrafted protein of any one of claims 1 to 3, wherein the IgG class light chain comprises 95% or more sequence identity with SEQ ID NO:69 and the IgG class heavy chain comprises 95% or more sequence identity with SEQ ID NO:53.

17. A pharmaceutical composition comprising the antibody cytokine engrafted protein of any one of claims 1 to 16 and a pharmaceutically acceptable carrier.

18. Use of the antibody cytokine engrafted protein of any one of claims 1 to 16 or the pharmaceutical composition of claim 17 in the manufacture of a medicament for the treatment of cancer.

19. The use of claim 18, wherein the cancer is selected from the group consisting of melanoma, lung cancer, colorectal cancer, prostate cancer, breast cancer, and lymphoma.

20. The use of claim 18 or 19 for combination therapy of cancer, wherein the antibody cytokine engrafted protein or pharmaceutical composition is to be administered in combination with a therapeutic agent.
21. The use of claim 20, wherein the therapeutic agent is a second antibody cytokine engrafted protein.
22. The use of claim 20, wherein the therapeutic agent is an immune checkpoint inhibitor.
23. The use of claim 22, wherein the immune checkpoint inhibitor is selected from the group consisting of: PD-1, PD-L1, PD-L2, TIM3, CTLA-4, LAG-3, CEACAM-1, CEACAM-5, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4 and TGFR.
24. The use of claim 20, wherein the therapeutic agent is a tyrosine kinase inhibitor.
25. Use of the antibody cytokine engrafted protein of any one of claims 1 to 16 or the pharmaceutical composition of claim 17 in the manufacture of a medicament for preferentially expanding T effector cells over regulatory T cells in a patient in need thereof.
26. The use of claim 25, wherein the T effector cells are expanded and Treg cells are not expanded.
27. The use of any one of claims 25 to 26, wherein an immune checkpoint inhibitor is to be administered.
28. The use of claim 27, wherein the immune checkpoint inhibitor is selected from the group consisting of: PD-1, PD-L1, PD-L2, TIM3, CTLA-4, LAG-3, CEACAM-1, CEACAM-5, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4 and TGFR.
29. The antibody cytokine engrafted protein of any one of claims 1 to 16 or the pharmaceutical composition of claim 17 for use in the treatment of cancer.

30. An isolated nucleic acid encoding an antibody cytokine engrafted protein comprising: (i) a heavy chain encoded by SEQ ID NO:22 and a light chain encoded by SEQ ID NO:38; or (ii) a heavy chain encoded by SEQ ID NO:54 and a light chain encoded by SEQ ID NO:70.
31. A recombinant host cell suitable for the production of an antibody cytokine engrafted protein, comprising the nucleic acid of claim 30 encoding the heavy and light chain polypeptides of the antibody cytokine engrafted protein, and optionally, a secretion signal.
32. The recombinant host cell of claim 31, which is a mammalian cell line.
33. The recombinant host cell of claim 32, wherein the mammalian cell line is a CHO cell line, Cos cell line, HeLa cell line, myeloma cell line, transformed B-cell line, or a hybridoma.