

CLAIMS

1. An immunocompetent cell that expresses a chimeric antigen receptor (CAR)~~a cell surface molecule~~ specifically recognizing human mesothelin,~~;~~ interleukin 7 (IL-7)~~;~~ and chemokine (C-C motif) ligand 19 (CCL19)~~;~~.

the CAR comprising a single chain antibody, a transmembrane region, and a signaling region that induces activation of the immunocompetent cell,

wherein the single chain antibody in the CAR is (1-1) a single chain antibody comprising a heavy chain variable region comprising heavy chain CDR1 consisting of the amino acid sequence shown by SEQ ID NO: 13, heavy chain CDR2 consisting of the amino acid sequence shown by SEQ ID NO: 14, and heavy chain CDR3 consisting of the amino acid sequence shown by SEQ ID NO: 15; and a light chain variable region comprising light chain CDR1 consisting of the amino acid sequence shown by SEQ ID NO: 16, light chain CDR2 consisting of the amino acid sequence shown by SEQ ID NO: 17, and light chain CDR3 consisting of the amino acid sequence shown by SEQ ID NO: 18.

2. The immunocompetent cell according to claim 1, wherein the immunocompetent cell is an immunocompetent cell separated from a living body.

3. The immunocompetent cell according to claim 1 or 2, wherein the immunocompetent cell comprises an exogenous nucleic acid encoding a ~~cell surface molecule~~CAR specifically recognizing human mesothelin, an exogenous nucleic acid encoding IL-7, and an exogenous nucleic acid encoding CCL19.

4. The immunocompetent cell according to claim 3, wherein the exogenous nucleic acid encoding IL-7, and the exogenous nucleic acid encoding CCL19 are an exogenous nucleic acid encoding human IL-7, and an exogenous nucleic acid encoding human CCL19.

5. The immunocompetent cell according to claim ~~3 or~~ 4, wherein the exogenous nucleic

acid encoding a ~~cell surface molecule~~ CAR specifically recognizing human mesothelin, the exogenous nucleic acid encoding human IL-7, and the exogenous nucleic acid encoding human CCL19 are integrated in a genome.

~~6. The immunocompetent cell according to any one of claims 1 to 5, wherein the cell surface molecule specifically recognizing human mesothelin is a chimeric antigen receptor (CAR) having a single chain antibody, a transmembrane region, and a signaling region that induces activation of the immunocompetent cell.~~

~~7. The immunocompetent cell according to claim 6, wherein the single chain antibody in the CAR is any of the following single chain antibodies:~~

~~(1-1) a single chain antibody comprising a heavy chain variable region comprising heavy chain CDR1 consisting of the amino acid sequence shown by SEQ ID NO: 13, heavy chain CDR2 consisting of the amino acid sequence shown by SEQ ID NO: 14, and heavy chain CDR3 consisting of the amino acid sequence shown by SEQ ID NO: 15, and a light chain variable region comprising light chain CDR1 consisting of the amino acid sequence shown by SEQ ID NO: 16, light chain CDR2 consisting of the amino acid sequence shown by SEQ ID NO: 17, and light chain CDR3 consisting of the amino acid sequence shown by SEQ ID NO: 18;~~

~~(2-1) a single chain antibody comprising a heavy chain variable region comprising heavy chain CDR1 consisting of the amino acid sequence shown by SEQ ID NO: 13, heavy chain CDR2 consisting of the amino acid sequence shown by SEQ ID NO: 14, and heavy chain CDR3 consisting of the amino acid sequence shown by SEQ ID NO: 15, and a light chain variable region comprising light chain CDR1 consisting of the amino acid sequence shown by SEQ ID NO: 19, light chain CDR2 consisting of the amino acid sequence shown by SEQ ID NO: 17, and light chain CDR3 consisting of the amino acid sequence shown by SEQ ID NO: 18; and~~

~~(3-1) a single chain antibody comprising a heavy chain variable region comprising heavy chain CDR1 consisting of the amino acid sequence shown by SEQ ID NO: 20, heavy chain CDR2 consisting of the amino acid sequence shown by SEQ ID NO: 21, and heavy chain CDR3 consisting of the amino acid sequence shown by SEQ ID NO: 22, and a light chain variable region comprising light chain CDR1 consisting of the amino~~

~~acid sequence shown by SEQ ID NO: 23, light chain CDR2 consisting of the amino acid sequence shown by SEQ ID NO: 24, and light chain CDR3 consisting of the amino acid sequence shown by SEQ ID NO: 25.~~

~~8. The immunocompetent cell according to claim 6 or 7, wherein the single chain antibody in the CAR is any of the following single chain antibodies:~~

6. The immunocompetent cell according to any one of claims 1 to 5, wherein the single chain antibody in the CAR is a single chain antibody comprising a heavy chain variable region consisting of an amino acid sequence having 85% or higher sequence identity to the amino acid sequence shown by SEQ ID NO:1, and comprising heavy chain CDR1 consisting of the amino acid sequence shown by SEQ ID NO: 13, heavy chain CDR2 consisting of the amino acid sequence shown by SEQ ID NO: 14, and heavy chain CDR3 consisting of the amino acid sequence shown by SEQ ID NO: 15; and a light chain variable region consisting of an amino acid sequence having 85% or higher sequence identity to the amino acid sequence shown by SEQ ID NO:2, ———and comprising light chain CDR1 consisting of the amino acid sequence shown by SEQ ID NO: 16, light chain CDR2 consisting of the amino acid sequence shown by SEQ ID NO: 17, and light chain CDR3 consisting of the amino acid sequence shown by SEQ ID NO: 18

~~(1-2) a single chain antibody comprising a heavy chain variable region consisting of an amino acid sequence having 85% or higher sequence identity to the amino acid sequence shown by SEQ ID NO: 1, and a light chain variable region consisting of an amino acid sequence having 85% or higher sequence identity to the amino acid sequence shown by SEQ ID NO: 2;~~

~~(2-2) a single chain antibody comprising a heavy chain variable region consisting of an amino acid sequence having 85% or higher sequence identity to the amino acid sequence shown by SEQ ID NO: 3, and a light chain variable region consisting of an amino acid sequence having 85% or higher sequence identity to the amino acid sequence shown by SEQ ID NO: 4;~~

~~(3-2) a single chain antibody comprising a heavy chain variable region consisting of an amino acid sequence having 85% or higher sequence identity to the amino acid~~

~~sequence shown by SEQ ID NO: 5, and a light chain variable region consisting of an amino acid sequence having 85% or higher sequence identity to the amino acid sequence shown by SEQ ID NO: 6;~~

~~(4-2) a single chain antibody comprising a heavy chain variable region consisting of an amino acid sequence having 85% or higher sequence identity to the amino acid sequence shown by SEQ ID NO: 1, and a light chain variable region consisting of an amino acid sequence having 85% or higher sequence identity to the amino acid sequence shown by SEQ ID NO: 4; and~~

~~(5-2) a single chain antibody comprising a heavy chain variable region consisting of an amino acid sequence having 85% or higher sequence identity to the amino acid sequence shown by SEQ ID NO: 3, and a light chain variable region consisting of an amino acid sequence having 85% or higher sequence identity to the amino acid sequence shown by SEQ ID NO: 2.~~

97. The immunocompetent cell according to any one of claims ~~6-1~~ to ~~86~~, wherein the single chain antibody in the CAR is ~~any of the following single chain antibodies:~~

~~(1-3) a single chain antibody comprising a heavy chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 1, and a light chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 2;~~

~~(2-3) a single chain antibody comprising a heavy chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 3, and a light chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 4;~~

~~(3-3) a single chain antibody comprising a heavy chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 5, and a light chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 6;~~

~~(4-3) a single chain antibody comprising a heavy chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 1, and a light chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 4; and~~

~~(5-3) a single chain antibody comprising a heavy chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 3, and a light chain variable region~~

~~consisting of the amino acid sequence shown by SEQ ID NO: 2.~~

~~108.~~ The immunocompetent cell according to any one of claims ~~6-1~~ to ~~97~~, wherein the transmembrane region in the CAR comprises an amino acid sequence having 85% or higher sequence identity to the amino acid sequence shown by SEQ ID NO: 7, and comprises a polypeptide that has action equivalent to that of the amino acid sequence shown by SEQ ID NO: 7.

9. The immunocompetent cell according to any one of claims 1 to 8, wherein the signaling region that induces activation of the immunocompetent cell in the CAR comprises:

(i) a polypeptide that comprises an amino acid sequence having 85% or higher sequence identity to the amino acid sequence shown by SEQ ID NO:9, and that has action equivalent to that of the amino acid sequence shown by SEQ ID NO: 9, or a polypeptide that consists of an amino acid sequence derived from the amino acid sequence shown by SEQ ID NO: 9 by the deletion, substitution, insertion, and/or addition of one or several amino acids, and that has action equivalent to that of the amino acid sequence shown by SEQ ID NO: 9; and

(ii) a polypeptide that comprises an amino acid sequence having 85% or higher sequence identity to the amino acid sequence shown by SEQ ID NO:10, and that has action equivalent to that of the amino acid sequence shown by SEQ ID NO: 10, or a polypeptide that consists of an amino acid sequence derived from the amino acid sequence shown by SEQ ID NO: 10 by the deletion, substitution, insertion, and/or addition of one or several amino acids, and that has action equivalent to that of the amino acid sequence shown by SEQ ID NO: 10.

~~110.~~ The immunocompetent cell according to ~~any one of claims 6 to 10~~claim 9, wherein the signaling region that induces ~~the~~ activation of the immunocompetent cell in the CAR further comprises the amino acid sequences shown by SEQ ID NOs: ~~8, 9 and 10.~~

~~12~~11. The immunocompetent cell according to any one of claims ~~7~~1 to ~~44~~10, wherein the heavy chain variable region and the light chain variable region are connected via a peptide linker consisting of a 2- to 30-amino acid sequence.

~~13~~12. The immunocompetent cell according to claim ~~12~~11, wherein the peptide linker consists of the amino acid sequence shown by SEQ ID NO: 26 or SEQ ID NO: 27.

13. The immunocompetent cell according to any one of claims 1 to 12, wherein the light chain variable region is positioned on a C-terminal side of the heavy chain variable region.

14. The immunocompetent cell according to any one of claims ~~6~~1 to 13, wherein the single chain antibody in the CAR is ~~any of the following single chain antibodies:~~

(1-4) a single chain antibody sequentially comprising a heavy chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 1, a peptide linker consisting of the amino acid sequence shown by SEQ ID NO: 26, and a light chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 2;

~~(2-4) a single chain antibody sequentially comprising a heavy chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 3, a peptide linker consisting of the amino acid sequence shown by SEQ ID NO: 26, and a light chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 4;~~

~~(3-4) a single chain antibody sequentially comprising a light chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 2, a peptide linker consisting of the amino acid sequence shown by SEQ ID NO: 26, and a heavy chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 1;~~

~~(4-4) a single chain antibody sequentially comprising a heavy chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 1, a peptide linker consisting of the amino acid sequence shown by SEQ ID NO: 27, and a light chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 2;~~

~~(5-4) a single chain antibody sequentially comprising a light chain variable region~~

~~consisting of the amino acid sequence shown by SEQ ID NO: 2, a peptide linker consisting of the amino acid sequence shown by SEQ ID NO: 27, and a heavy chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 1;~~
~~(6-4) a single chain antibody sequentially comprising a heavy chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 5, a peptide linker consisting of the amino acid sequence shown by SEQ ID NO: 26, and a light chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 6;~~
~~(7-4) a single chain antibody sequentially comprising a light chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 6, a peptide linker consisting of the amino acid sequence shown by SEQ ID NO: 26, and a heavy chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 5;~~
~~(8-4) a single chain antibody sequentially comprising a heavy chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 5, a peptide linker consisting of the amino acid sequence shown by SEQ ID NO: 27, and a light chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 6; and~~
~~(9-4) a single chain antibody sequentially comprising a light chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 6, a peptide linker consisting of the amino acid sequence shown by SEQ ID NO: 27, and a heavy chain variable region consisting of the amino acid sequence shown by SEQ ID NO: 5.~~

15. The immunocompetent cell according to any one of claims 1 to 14, wherein the signaling region that induces activation of the immunocompetent cell in the CAR comprises a polypeptide of a 4-1BB intracellular region, and a polypeptide of a CD3 ζ intracellular region.

~~15~~16. The immunocompetent cell according to ~~any one of claims 6 to 14~~claim 15, wherein the signaling region that induces ~~the~~ activation of the immunocompetent cell in the CAR further comprises a polypeptide of a CD28 intracellular region,~~a polypeptide of a 4-1BB intracellular region, and a polypeptide of a CD3 ζ intracellular region.~~

~~16~~17. The immunocompetent cell according to any one of claims 1 to ~~15~~16, wherein the

immunocompetent cell is a T cell.

~~17~~18. The immunocompetent cell according to any one of claims 1 to ~~16~~17, wherein the immunocompetent cell is a T cell derived from a human or ~~is a T cell~~ separated from a human.

~~18~~19. A pharmaceutical composition comprising ~~an~~the immunocompetent cell according to any one of claims 1 to ~~17~~18 and a pharmaceutically acceptable additive.

~~19~~20. The pharmaceutical composition according to claim ~~18~~19 for use in the treatment of a cancer- type expressing mesothelin in a cancer tissue, or a cancer type derived from cancer cells expressing mesothelin-cancer.

~~20~~21. An expression vector comprising a nucleic acid encoding a chimeric antigen receptor (CAR) ~~a cell surface molecule~~ specifically recognizing human mesothelin, a nucleic acid encoding IL-7, and a nucleic acid encoding CCL19, the CAR comprising a single chain antibody, a transmembrane region, and a signaling region that induces activation of the immunocompetent cell, wherein the single chain antibody in the CAR is (1-1) a single chain antibody comprising a heavy chain variable region comprising heavy chain CDR1 consisting of the amino acid sequence shown by SEQ ID NO: 13, heavy chain CDR2 consisting of the amino acid sequence shown by SEQ ID NO: 14, and heavy chain CDR3 consisting of the amino acid sequence shown by SEQ ID NO: 15; and a light chain variable region comprising light chain CDR1 consisting of the amino acid sequence shown by SEQ ID NO: 16, light chain CDR2 consisting of the amino acid sequence shown by SEQ ID NO: 17, and light chain CDR3 consisting of the amino acid sequence shown by SEQ ID NO: 18.

~~21~~22. A method for producing an immunocompetent cell that expresses a chimeric antigen receptor (CAR) ~~a cell surface molecule~~ specifically recognizing human mesothelin, IL-7, and CCL19, comprising introducing a nucleic acid encoding the ~~cell surface molecule~~CAR specifically recognizing human mesothelin, a nucleic acid encoding the IL-7, and a nucleic acid encoding the CCL19 to an immunocompetent cell, the CAR comprising a single chain antibody, a transmembrane region, and a signaling region that induces activation of the immunocompetent cell.

wherein the single chain antibody in the CAR is (1-1) a single chain antibody comprising a heavy chain variable region comprising heavy chain CDR1 consisting of the amino acid sequence shown by SEQ ID NO: 13, heavy chain CDR2 consisting of the amino acid sequence shown by SEQ ID NO: 14, and heavy chain CDR3 consisting of the amino acid sequence shown by SEQ ID NO: 15; and a light chain variable region comprising light chain CDR1 consisting of the amino acid sequence shown by SEQ ID NO: 16, light chain CDR2 consisting of the amino acid sequence shown by SEQ ID NO: 17, and light chain CDR3 consisting of the amino acid sequence shown by SEQ ID NO: 18.