

CLAIMS

1. An isolated antibody or antigen binding fragment that specifically binds mesothelin, comprising:

i. a single domain antibody (sdAb) comprising a complementarity determining region 1 (CDR1), CDR2 and CDR3 having the polypeptide sequences of:

- a. SEQ ID NOs:66, 134, and 202, respectively;
- b. SEQ ID NOs:67, 135, and 203, respectively;
- c. SEQ ID NOs:34, 102, and 170, respectively;
- d. SEQ ID NOs:54, 122, and 190, respectively;
- e. SEQ ID NOs:55, 123, and 191, respectively;
- f. SEQ ID NOs:61, 129, and 197, respectively;
- ~~g. SEQ ID NOs:31, 99, and 167, respectively;~~
- ~~h. SEQ ID NOs:32, 100, and 168, respectively;~~
- ~~i. SEQ ID NOs:33, 101, and 169, respectively;~~
- ~~j. SEQ ID NOs:35, 103, and 171, respectively;~~
- ~~k. SEQ ID NOs:36, 104, and 172, respectively;~~
- ~~l. SEQ ID NOs:37, 105, and 173, respectively;~~
- ~~m. SEQ ID NOs:38, 106, and 174, respectively;~~
- ~~n. SEQ ID NOs:39, 107, and 175, respectively;~~
- ~~o. SEQ ID NOs:40, 108, and 176, respectively;~~
- ~~p. SEQ ID NOs:41, 109, and 177, respectively;~~
- ~~q. SEQ ID NOs:42, 110, and 178, respectively;~~
- ~~r. SEQ ID NOs:43, 111, and 179, respectively;~~
- ~~gs. SEQ ID NOs:44, 112, and 180, respectively;~~
- ~~ht. SEQ ID NOs:45, 113, and 181, respectively;~~
- ~~u. SEQ ID NOs:46, 114, and 182, respectively;~~
- ~~v. SEQ ID NOs:47, 115, and 183, respectively;~~
- ~~iw. SEQ ID NOs:48, 116, and 184, respectively;~~
- ~~x. SEQ ID NOs:49, 117, and 185, respectively;~~
- ~~y. SEQ ID NOs:50, 118, and 186, respectively;~~
- ~~z. SEQ ID NOs:51, 119, and 187, respectively;~~
- ~~aa. SEQ ID NOs:52, 120, and 188, respectively;~~
- ~~jbb. SEQ ID NOs:53, 121, and 189, respectively;~~or
- ~~kee. SEQ ID NOs:56, 124, and 192, respectively;~~

~~dd. SEQ ID NOs:57, 125, and 193, respectively;~~
~~ee. SEQ ID NOs:58, 126, and 194, respectively;~~
~~ff. SEQ ID NOs:59, 127, and 195, respectively;~~
~~gg. SEQ ID NOs:60, 128, and 196, respectively;~~
~~hh. SEQ ID NOs:62, 130, and 198, respectively;~~
~~ii. SEQ ID NOs:63, 131, and 199, respectively;~~
~~jj. SEQ ID NOs:64, 132, and 200, respectively;~~
~~kk. SEQ ID NOs:65, 133, and 201, respectively; or~~
~~ll. SEQ ID NOs:68, 136, and 204, respectively; or~~

ii. a single chain variable fragment (scFv) comprising a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, and a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of:

a. SEQ ID NOs:1, 69, 137, 2, 70, and 138, respectively;
~~b. SEQ ID NOs:19, 87, 155, 20, 88, and 156, respectively;~~
be. SEQ ID NOs:23, 91, 159, 24, 92, and 160, respectively;
cd. SEQ ID NOs:25, 93, 161, 26, 94, and 162, respectively;
de. SEQ ID NOs:27, 95, 163, 28, 96, and 164, respectively;
ef. SEQ ID NOs:29, 97, 165, 30, 98, and 166, respectively;
~~g. SEQ ID NOs:3, 71, 139, 4, 72, and 140, respectively;~~
~~h. SEQ ID NOs:5, 73, 141, 6, 74, and 142, respectively;~~
fi. SEQ ID NOs:7, 75, 143, 8, 76, and 144, respectively; or
~~_____gj. SEQ ID NOs:9, 77, 145, 10, 78, and 146, respectively;~~
~~k. SEQ ID NOs:11, 79, 147, 12, 80, and 148, respectively;~~
l. SEQ ID NOs:13, 81, 149, 14, 82, and 150, respectively;
m. SEQ ID NOs:15, 83, 151, 16, 84, and 152, respectively;
n. SEQ ID NOs:17, 85, 153, 18, 86, and 154, respectively; or
o. SEQ ID NOs:21, 89, 157, 22, 90, and 158, respectively.

2. The isolated antibody or antigen binding fragment of claim 1, wherein

i. the single domain antibody comprises an amino acid sequence at least 95% identical to the amino acid sequence selected from the group consisting of SEQ ID NOs: 224, 234-235, 238, 243-246, 251, 256-257~~221-258~~ and SEQ ID NOs: 420-428, or

ii. the single chain variable fragment comprises an amino acid sequence at least 95% identical to the amino acid sequence selected from the group consisting of SEQ ID NOs: 205-206, 209-210, 217-220~~205-220~~.

3. An isolated polynucleotide comprising a nucleotide sequence encoding a chimeric antigen receptor (CAR), wherein the CAR comprises:

(a) an extracellular domain comprising an antigen binding fragment that comprises the isolated antibody or antigen binding fragment of claim 1 or 2;

(b) a transmembrane domain; and

(c) an intracellular signaling domain,

wherein the CAR optionally further comprises a signal peptide at the amino terminus and a hinge region connecting the extracellular domain and the transmembrane domain.

4. The isolated polynucleotide of claim 3, wherein the CAR comprises the amino acid sequence selected from the group consisting of SEQ ID NOs: 358, 360-367~~358-367~~ and SEQ ID NOs:445-447.

5. An isolated polynucleotide comprising a nucleotide sequence encoding a chimeric antigen receptor (CAR), wherein the CAR comprises:

(a) an extracellular domain comprising a first polypeptide that binds specifically to a follicle-stimulating hormone receptor (FSHR) and an antigen binding fragment that comprises the isolated antibody or antigen binding fragment of claim 1 or 2;

(b) a transmembrane domain; and

(c) an intracellular signaling domain,

wherein the CAR optionally further comprises a signal peptide at the amino terminus and a hinge region connecting the extracellular domain and the transmembrane domain.

6. The isolated polynucleotide of claim 5, wherein ~~extracellular domain of the CAR comprises the amino acid sequence selected from the group consisting of SEQ ID NOs:348-357, and/or the CAR comprises the amino acid sequence selected from the group consisting of SEQ ID NO:370-379 and~~ SEQ ID NOs: 375, 448-450.

7. The isolated polynucleotide of claim 3, further comprising a second nucleotide sequence encoding a second chimeric antigen receptor (CAR), wherein the second CAR comprises:

(a) an extracellular domain comprising a polypeptide that binds specifically to a follicle-stimulating hormone receptor (FSHR);

(b) a transmembrane domain; and

(c) an intracellular signaling domain,

wherein the CAR optionally further comprises a signal peptide at the amino terminus and a hinge region connecting the extracellular domain and the transmembrane domain, preferably the nucleotide sequence encoding the CAR is connected to the second nucleotide sequence via a 2A peptide coding sequence.

8. The isolated polynucleotide of claim 7, wherein the polypeptide that binds specifically to FSHR comprises the amino acid sequence selected from the group consisting of SEQ ID NOs: 319-331, and/or the isolated polynucleotide ~~comprises~~encodes the amino acid sequence selected from the group consisting of ~~SEQ ID NOs: 380-389 and~~ SEQ ID NOs: 385, 451-453.

9. The isolated polynucleotide of claim 3, further comprising a second nucleotide sequence encoding a fusion protein comprising, from the N-terminus to the C-terminus, a polypeptide that binds specifically to a follicle-stimulating hormone receptor (FSHR), and an extracellular domain, a transmembrane domain and an intracellular domain of a CD3 polypeptide selected from the group consisting of a CD3-γ, CD3-δ and CD3-ε chain, preferably the nucleotide sequence encoding the CAR is connected to the second nucleotide sequence via a 2A peptide coding sequence.

10. The isolated polynucleotide of claim 9, wherein the isolated polynucleotide ~~comprise~~encodes the amino acid sequence selected from the group consisting of SEQ ID NOs: 454-456.

11. The isolated polynucleotide of any one of claims 3-10, further comprising an third additional nucleotide sequence encoding a ~~dominant negative form of~~ an inhibitor of a cell-mediated immune response of the immune cell, preferably the nucleotide sequence encoding the CAR or the second CAR is connected to the ~~third~~third additional nucleotide sequence via a 2A peptide coding sequence.

12. The isolated polynucleotide of claim 11, wherein the inhibitor of a cell-mediated immune response of the immune cell is a dominant negative form of a transforming growth factor β (TGF- β) receptor.

13. The isolated polynucleotide of claim 12, wherein the ~~dominant negative form of the~~ inhibitor comprises the amino acid sequence of SEQ ID NO: 347.

14. The isolated polynucleotide of claim 13, wherein the isolated polynucleotide encodes a protein having the amino acid sequence selected from the group consisting of SEQ ID NOs: ~~390-399, 400-409, 410-419, 460-462, 463-465, 466-468 and 469-471.~~

15. The isolated polynucleotide of any one of claims 3-14, wherein the transmembrane domain is selected from the group consisting of a CD8 α transmembrane domain, a CD28 transmembrane domain, a CD4 transmembrane domain, a CD3 ζ transmembrane domain, a CD2 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, a BTLA transmembrane domain, and a GMCSFR transmembrane domain;
the intracellular signaling domain is selected from the group consisting of a signaling domain of CD3 ζ , FcR γ , FcR β , CD3 γ , CD3 δ , CD3 ϵ , CD5, CD22, CD79a, CD79b, and CD66d;
wherein the CAR optionally further comprises a co-stimulatory domain selected from the group consisting of a co-stimulatory domain of one or more of CD28, 4-1BB (CD137), CD27, OX40, CD27, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, TNFRSF9, TNFRSF4, TNFRSF8, CD40LG, ITGB2, KLRC2, TNFRSF18, TNFRSF14, HAVCR1, LGALS9, CD83, and a ligand that specifically binds with CD83.

16. An isolated nucleic acid encoding the isolated antibody or antigen binding fragment thereof of claim 1 or 2.

17. An engineered immune cell expressing the CAR ~~or TCR complex~~ encoded by the isolated polynucleotide of any of claims 3-15.

18. The engineered immune cell of claim 17, wherein the engineered immune cell is selected from the group consisting of a cytotoxic T cell, a helper T cell, a natural killer T cell, a $\gamma\delta$ T cell, and a NK cell.

19. A pharmaceutical composition, comprising the isolated antibody or antigen binding fragment thereof of any one of claims 1 or 2, the isolated polynucleotide of any one of claims 3-15, or the engineered immune cell of claim 17 or 18, and a pharmaceutically acceptable carrier.

20. Use of an effective amount of the pharmaceutical composition of claim 19 in manufacture of a medicament for treating ~~a cancer, preferably an ovarian cancer, primary peritoneal carcinomas, pancreatic ductal adenocarcinoma (PDA), malignant pleural mesothelioma (MPM), lung adenocarcinoma, triple negative breast cancer, endometrial cancer, biliary cancer, gastric cancer, or pediatric acute myeloid leukemia.~~