CLAIMS

1. An isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), wherein the CAR comprises: i) an antibody or antibody fragment comprising a human anti-mesothelin binding domain, ii) a transmembrane domain, and iii) an intracellular signaling domain comprising a stimulatory domain, and wherein said anti-mesothelin binding domain:
   (a) comprises a light chain complementarity determining region 1 (LC CDR1), a light chain complementarity determining region 2 (LC CDR2), a light chain complementarity determining region 3 (LC CDR3), a heavy chain complementarity determining region 1 (HC CDR1), a heavy chain complementarity determining region 2 (HC CDR2), and a heavy chain complementarity determining region 3 (HC CDR3) of anti-mesothelin scFv M5 or M11 (Tables 4 and 5);
   (b) competes for binding to human mesothelin with an antigen binding domain comprising a sequence selected from SEQ ID NO: 43 and SEQ ID NO: 49;
   (c) binds an epitope within amino acids 450-588 of SEQ ID NO: 278; or
   (d) binds an epitope within amino acids 450-588 of SEQ ID NO: 278, wherein the epitope comprises amino acids 485-490, 498-507, 532-537, or 545-572 of SEQ ID NO: 278, or any subset or a combination thereof,

   optionally wherein the anti-mesothelin binding domain is a scFv.

2. The isolated nucleic acid molecule of claim 1, comprising a LC CDR1, a LC CDR2, a LC CDR3, a HC CDR1, a HC CDR2 and a HC CDR3 of anti-mesothelin scFv M5 or M11 (Tables 4 and 5).

3. The isolated nucleic acid molecule of claim 1, wherein the anti-mesothelin binding domain comprises:
   (i) the amino acid sequence of a light chain variable region of anti-mesothelin scFv M5 or M11 listed in Table 2;
   (ii) the amino acid sequence of a heavy chain variable region of anti-mesothelin scFv M5 or M11 listed in Table 2;
   (iii) the amino acid sequence of a light chain variable region and a heavy chain variable region of anti-mesothelin scFv M5 or M11 listed in Table 2;
   (iv) a light chain variable region comprising an amino acid sequence having at least one, two or three modifications but not more than 30, 20 or 10 modifications of an amino acid sequence of a light chain variable region of anti-mesothelin scFv M5 or M11 provided in Table 2;

(v) an amino acid sequence with 95-99% identity to the amino acid sequence of a light chain variable region of anti-mesothelin scFv M5 or M11 provided in Table 2;

(vi) a heavy chain variable region comprising an amino acid sequence having at least one, two or three modifications but not more than 30, 20 or 10 modifications of the amino acid sequence of a heavy chain variable region of anti-mesothelin scFv M5 or M11 provided in Table 2; or

(vii) an amino acid sequence with 95-99% identity to the amino acid sequence of a heavy chain variable region of anti-mesothelin scFv M5 or M11 provided in Table 2.

4. The isolated nucleic acid molecule of any of the preceding claims, wherein

a) the encoded anti-mesothelin binding domain comprises the amino acid sequence of SEQ ID NO: 43 or SEQ ID NO: 49, or a sequence with 95-99% identity to the amino acid sequence of SEQ ID NO: 43 or SEQ ID NO: 49; or

b) the nucleic acid sequence encoding the anti-mesothelin binding domain comprises the nucleic acid sequence of SEQ ID NO: 91 or SEQ ID NO: 97, or a sequence with 95-99% identity to the nucleic acid sequence of SEQ ID NO: 91 or SEQ ID NO: 97.

5. The isolated nucleic acid molecule of any of the preceding claims, wherein

a) the encoded CAR includes a transmembrane domain that comprises a transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154;

b) the encoded transmembrane domain comprises the amino acid sequence of SEQ ID NO: 6;

c) the encoded transmembrane domain comprises an amino acid sequence that comprises at least one, two or three modifications but not more than 20, 10 or 5 modifications of the amino acid sequence of SEQ ID NO:6;

d) the encoded transmembrane domain comprises an amino acid sequence with 95-99% identity to the amino acid sequence of SEQ ID NO:6; or

e) the nucleic acid sequence encoding the transmembrane domain comprises the nucleic acid sequence of SEQ ID NO:17, or a sequence with 95-99% identity to the nucleic acid sequence of SEQ ID NO:17.

6. The isolated nucleic acid molecule of any of the preceding claims, wherein the encoded anti-mesothelin binding domain is connected to the transmembrane domain by a hinge region, wherein

a) the encoded hinge region comprises the amino acid sequence of SEQ ID NO:2, or the encoded hinge region comprises a sequence with 95-99% identity to the amino acid sequence of SEQ ID NO:2, or

b) the encoded hinge region comprises the amino acid sequence encoded by SEQ ID NO: 13, or the encoded hinge region comprises an amino acid sequence encoded by a sequence with 95-99% identity to the nucleic acid sequence of SEQ ID NO:13.

7. The isolated nucleic acid molecule of any of the preceding claims, further comprising a sequence encoding a costimulatory domain, wherein

a) the encoded costimulatory domain is a functional signaling domain derived from a protein selected from the group consisting of OX40, CD2, CD27, CD28, CDS, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), and 4-1BB (CD137);

b) the encoded costimulatory domain comprises the amino acid sequence of SEQ ID NO:7;

c) the encoded costimulatory domain comprises an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of the amino acid sequence of SEQ ID NO:7;

d) the encoded costimulatory domain comprises a sequence with 95-99% identity to the amino acid sequence of SEQ ID NO:7;

e) the nucleic acid sequence encoding the costimulatory domain comprises the nucleic acid sequence of SEQ ID NO:18;

f) the sequence encoding the costimulatory domain comprises a sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of the nucleic acid sequence of SEQ ID NO:18; or

g) the sequence encoding the costimulatory domain comprises a sequence with 95-99% identity to the nucleic acid sequence of SEQ ID NO:18.

8. The isolated nucleic acid molecule of any of the preceding claims, wherein the encoded intracellular signaling domain comprises a functional signaling domain of 4-1BB and/or a functional signaling domain of CD3 zeta, optionally wherein the encoded intracellular signaling domain comprises:

(i) the amino acid sequence of SEQ ID NO: 7 and the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10;

(ii) an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of the amino acid sequence of SEQ ID NO:7 and the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10;

(iii) an amino acid sequence with 95-99% identity to the amino acid sequence of SEQ ID NO:7 and the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10;

(iv) the amino acid sequence of SEQ ID NO:7 and the sequence of SEQ ID NO:9 or SEQ ID NO:10, wherein the sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide chain; or

(v) the amino acid sequence encoded by the sequence of SEQ ID NO:18, or a sequence with 95-99% identity thereto, and the amino acid sequence encoded by the sequence of SEQ ID NO:20 or SEQ ID NO:21, or a sequence with 95-99% identity thereto.

9. The isolated nucleic acid molecule of any of the preceding claims, further comprising a leader sequence wherein the leader sequence optionally comprises the amino acid sequence of SEQ ID NO: 1.

10. An isolated chimeric antigen receptor (CAR) molecule encoded by the nucleic acid molecule of any one of claims 1-9, optionally wherein, the CAR comprises the amino acid sequence of SEQ ID NO: 67, or SEQ ID NO: 73.

11. An isolated chimeric antigen receptor (CAR) molecule comprising: i) an antibody or antibody fragment which includes a human anti-mesothelin binding domain, ii) a transmembrane domain, and iii) an intracellular signaling domain, wherein the anti-mesothelin binding domain (e.g., a scFv):

(a) comprises a light chain complementarity determining region 1 (LC CDR1), a light chain complementarity determining region 2 (LC CDR2), a light chain complementarity determining region 3 (LC CDR3), a heavy chain complementarity determining region 1 (HC CDR1), a heavy chain complementarity determining region 2 (HC CDR2), and a heavy chain complementarity determining region 3 (HC CDR3) of anti-mesothelin scFv M5 or M11 (Tables 4 and 5);

(b) competes for binding to human mesothelin with an antigen binding domain comprising a sequence selected from SEQ ID NO: 43 and SEQ ID NO: 49;

(c) binds an epitope within amino acids 450-588 of SEQ ID NO: 278; or

(d) binds an epitope within amino acids 450-588 of SEQ ID NO: 278, wherein the epitope comprises amino acids 485-490, 498-507, 532-537, or 545-572 of SEQ ID NO: 278, or any subset or a combination thereof.

12. The isolated CAR molecule of claim 11, wherein the human anti-mesothelin binding domain (e.g., a scFv) comprises:

(i) LC CDR1, LC CDR2, LC CDR3, HC CDR1, HC CDR2, and HC CDR3 of anti-mesothelin scFv M5 or M11 (Table 4 and 5);

(ii) a light chain and a heavy chain of the amino acid sequence of anti-mesothelin scFv M5 or M11 listed in Table 2;

(iii) a light chain variable region comprising an amino acid sequence having at least one, two or three modifications but not more than 30, 20 or 10 modifications of the amino acid sequence of a light chain variable region of anti-mesothelin scFv M5 or M11 provided in Table 2;

(iv) a light chain variable region comprising an amino acid sequence with 95-99% identity with the amino acid sequence of anti-mesothelin scFv M5 or M11 provided in Table 2;

(v) a heavy chain variable region comprising an amino acid sequence having at least one, two or three modifications but not more than 30, 20 or 10 modifications of the amino acid sequence of a heavy chain variable region of anti-mesothelin scFv M5 or M11 provided in Table 2;

(vi) a heavy chain variable region comprising an amino acid sequence with 95-99% identity to the amino acid sequence of anti-mesothelin scFv M5 or M11 provided in Table 2;

(vii) the amino acid sequence of SEQ ID NO: 43 or SEQ ID NO: 49, or an amino acid sequence with 95-99% identity thereto; or

(viii) the amino acid sequence of SEQ ID NO: 67 or SEQ ID NO: 73., or an amino acid sequence with 95-99% identity thereto.

13. The isolated CAR molecule of claims 11 or 12, wherein the transmembrane domain comprises:

(i) a transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154;

(ii) the amino acid sequence of SEQ ID NO: 6;

(iii) an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of the amino acid sequence of SEQ ID NO: 6; or

(iv) an amino acid sequence with 95-99% identity to the amino acid sequence of SEQ ID NO: 6.

14. The isolated CAR molecule of any one of claims 11-13, wherein the human anti-mesothelin binding domain is connected to the transmembrane domain by a hinge region, wherein the hinge region comprises SEQ ID NO:2 or SEQ ID NO:36, or a sequence with 95-99% identity thereto.

15. The isolated CAR molecule of any of claims 11-14, wherein the intracellular signaling domain comprises a costimulatory domain, wherein the costimulatory domain comprises:

(a) a functional signaling domain of a protein selected from the group consisting of OX40, CD2, CD27, CD28, CDS, ICAM-1, LFA-1 (CD11a/CD18), CD278 (ICOS) and 4-1BB (CD137);

(b) the amino acid sequence of SEQ ID NO: 7;

(c) an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of the amino acid sequence of SEQ ID NO: 7; or

(d) an amino acid sequence with 95-99% identity to the amino acid sequence of SEQ ID NO: 7.

16. The isolated CAR molecule of any of claims 11-15, wherein the intracellular signaling domain comprises a functional signaling domain of 4-1BB and a functional signaling domain of CD3 zeta.

17. The isolated CAR molecule of claim 16, wherein the intracellular signaling domain comprises:

(i) the amino acid sequence of SEQ ID NO: 7 and the amino acid sequence of SEQ ID NO:9;

(ii) the amino acid sequence of SEQ ID NO:7 and the amino acid sequence of SEQ ID NO:10;

(iii) an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of the amino acid sequence of SEQ ID NO:7 and the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10;

(iv) an amino acid sequence with 95-99% identity to the amino acid sequence of SEQ ID NO:7 and the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10; or

(v) the amino acid sequence of SEQ ID NO: 7 and the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO:10, wherein the sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide chain.

18. The isolated CAR molecule of any of claims 11-17, further comprising a leader sequence, wherein the leader sequence optionally comprises the amino acid sequence of SEQ ID NO: 1, or a sequence with 95-99% identity to the amino acid sequence of SEQ ID NO:1.

19. A human anti-mesothelin binding domain, which:

(a) comprises a light chain complementarity determining region 1 (LC CDR1), a light chain complementarity determining region 2 (LC CDR2), a light chain complementarity determining region 3 (LC CDR3), a heavy chain complementarity determining region 1 (HC CDR1), a heavy chain complementarity determining region 2 (HC CDR2), and a heavy chain complementarity determining region 3 (HC CDR3) of a human anti-mesothelin binding domain in SEQ ID NO: 43, or SEQ ID NO: 49;

(b) competes for binding to human mesothelin with an antigen binding domain comprising a sequence selected from SEQ ID NO: 43 and SEQ ID NO: 49;

(c) binds an epitope within amino acids 450-588 of SEQ ID NO: 278; or

(d) binds an epitope within amino acids 450-588 of SEQ ID NO: 278, wherein the epitope comprises amino acids 485-490, 498-507, 532-537, or 545-572 of SEQ ID NO: 278, or any subset or a combination thereof.

20. The human anti-mesothelin binding domain of claim 19, wherein the human anti-mesothelin binding domain comprises a LC CDR1, a LC CDR2, a LC CDR3, a HC CDR1, a HC CDR2, and a HC CDR3 of a human anti-mesothelin binding domain in SEQ ID NO: 43 or SEQ ID NO: 49.

21. The human anti-mesothelin binding domain of claim 19, wherein:

(i) the human anti-mesothelin binding domain is a scFv comprising a light chain and a heavy chain of the amino acid sequence of SEQ ID NO: 43 or SEQ ID NO: 49; or

(ii) the human anti-mesothelin binding domain comprises: a light chain variable region comprising an amino acid sequence having at least one, two or three modifications but not more than 30, 20 or 10 modifications of the amino acid sequence of a light chain variable region provided in SEQ ID NO: 43 or SEQ ID NO: 49, or a sequence with 95-99% identity with the amino acid sequence of SEQ ID NO: 43 or SEQ ID NO: 49; and/or a heavy chain variable region comprising an amino acid sequence having at least one, two or three modifications but not more than 30, 20 or 10 modifications of the amino acid sequence of a heavy chain variable region provided in SEQ ID NO: 43 or SEQ ID NO: 49; or a sequence with 95-99% identity to the amino acid sequence in SEQ ID NO: 43 or SEQ ID NO: 49.

22. A vector comprising the nucleic acid molecule of any of claims 1-9, wherein the vector is selected from the group consisting of a DNA, a RNA, a plasmid, a lentivirus vector, adenoviral vector, or a retrovirus vector.

23. An immune effector cell comprising the vector of claim 22, wherein the immune effector cell is a human T cell (e.g., CD8+ T cell) or an NK cell.

24. A method of making an immune effector cell comprising transducing an immune effector cell with a vector of claim 22.

25. A method of generating a population of RNA-engineered cells comprising introducing an in vitro transcribed RNA or synthetic RNA into a cell, where the RNA comprises a nucleic acid molecule of any one of claims 1-9.

26. A cell comprising a nucleic acid molecule of any one of claims 1-9, for use in a method of providing an anti-cancer immunity in a mammal.

27. Use of a cell comprising a nucleic acid molecule of any one of claims 1-9, in the manufacture of a medicament for the provision of an anti-cancer immunity in a mammal.

28. A cell comprising a nucleic acid molecule of any one of claims 1-9, for use in a method of treating a mammal having a disease associated with expression of mesothelin, wherein the disease is selected from a proliferative disease such as a cancer or malignancy, or a

precancerous condition, or is a non-cancer related indication associated with expression of mesothelin.

29. Use of the isolated nucleic acid molecule of any of claims 1-9, the isolated CAR molecule of any of claims 10-18, the anti-mesothelin binding domain of any of claims 19-21, the vector of claim 22 or the immune effector cell of claim 23, in the manufacture of a medicament for treating a disease associated with expression of mesothelin, wherein the disease is selected from a proliferative disease such as a cancer or malignancy, or a precancerous condition, or is a non-cancer related indication associated with expression of mesothelin.

30. Use of a cell comprising a nucleic acid molecule of any one of claims 1-9 in the manufacture of a medicament for the treatment of a disease associated with expression of mesothelin, wherein the disease is a cancer associated with expression of mesothelin selected from the group consisting of mesothelioma, malignant pleural mesothelioma, non-small cell lung cancer, small cell lung cancer, squamous cell lung cancer, or large cell lung cancer, pancreatic cancer, pancreatic ductal adenocarcinoma, metastatic pancreatic cancer, ovarian cancer, colorectal cancer and bladder cancer, or any combination thereof.

31. The use according to claim 29, wherein the cancer is associated with expression of mesothelin selected from the group consisting of mesothelioma, malignant pleural mesothelioma, non-small cell lung cancer, small cell lung cancer, squamous cell lung cancer, or large cell lung cancer, pancreatic cancer, pancreatic ductal adenocarcinoma, metastatic pancreatic cancer, ovarian cancer, colorectal cancer and bladder cancer, or any combination thereof.

32. An immune effector cell of claim 23, further expressing an agent that inhibits a molecule that inhibits T cell function, wherein the agent comprises:
a first polypeptide that comprises an inhibitory molecule, wherein the inhibitory molecule is PD1, PD-L1, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4 or TGFR beta, or a fragment thereof; and
a second polypeptide that provides a positive signal to the cell, wherein the second polypeptide provides an intracellular signaling domain, further wherein the intracellular signaling domain comprises a co-stimulatory domain (e.g., 41BB, CD27 or CD28), and/or a primary signaling domain (e.g., CD3 zeta signaling domain).

33. Use of a cell comprising a nucleic acid molecule of any one of claims 1-9 in the manufacture of a medicament for the treatment of a disease associated with expression of mesothelin in a subject, wherein the disease is selected from a proliferative disease such as a cancer or malignancy, or a precancerous condition, or is a non-cancer related indication associated with expression of mesothelin, wherein the nucleic acid is to be introduced into T cells or NK cells using in vitro transcription, and the subject is to receive an initial administration of the cells comprising the nucleic acid, and one or more subsequent administrations of cells comprising the nucleic acid, wherein the one or more subsequent administrations are to be administered less than 15 days, e.g., 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, or 2 days after the previous administration.