

cytokines. These fusion proteins and methods of their use represent an advantage for T-cell receptor (TCR) fusion proteins (TFPs) relative to CARs because elevated levels of these cytokines have been associated with dose-limiting toxicities for adoptive CAR-T therapies.

SUMMARY OF THE INVENTION

[0005] Provided herein are T-cell receptor (TCR) fusion proteins (TFPs), T-cells engineered to express one or more TFPs, and methods of use thereof for the treatment of diseases.

[0006] In one aspect, provided herein is an isolated recombinant nucleic acid molecule encoding a T-cell receptor (TCR) fusion protein (TFP) comprising a TCR subunit and a human or humanized antibody domain comprising an anti-mesothelin binding domain.

[0006a] In one aspect, provided herein is a recombinant nucleic acid molecule encoding a T cell receptor (TCR) fusion protein (TFP) comprising

(a) a TCR subunit comprising (i) at least a portion of a TCR extracellular domain, (ii) a TCR transmembrane domain, and (iii) a TCR intracellular domain comprising a stimulatory domain from an intracellular signaling domain of CD3 epsilon, CD3 delta or CD3 gamma; and

(b) a human or humanized antibody domain comprising an antigen binding domain that is an anti-mesothelin binding domain,

wherein the TCR subunit and the human or humanized antibody domain are operatively linked, and wherein the TFP functionally interacts with a TCR when expressed in a T cell.

[0007] In one aspect, provided herein is an isolated recombinant nucleic acid molecule encoding a T-cell receptor (TCR) fusion protein (TFP) comprising a TCR subunit comprising at least a portion of a TCR extracellular domain, and a TCR intracellular domain comprising a stimulatory domain from an intracellular signaling domain of CD3 epsilon; and a human or humanized antibody domain comprising an antigen binding domain wherein the TCR subunit and the antibody domain are operatively linked, and wherein the TFP incorporates into a TCR when expressed in a T-cell.

[0008] In one aspect, provided herein is an isolated recombinant nucleic acid molecule encoding a T-cell receptor (TCR) fusion protein (TFP) comprising a TCR subunit comprising at least a portion of a TCR extracellular domain, and a TCR intracellular domain comprising a stimulatory domain from an intracellular signaling domain of CD3 gamma; and a human or humanized antibody domain comprising an antigen binding domain wherein the TCR subunit and the antibody domain are operatively linked, and wherein the TFP incorporates into a TCR when expressed in a T-cell.

[0009] In one aspect, provided herein is an isolated recombinant nucleic acid molecule encoding a T-cell receptor (TCR) fusion protein (TFP) comprising a TCR subunit comprising at least a portion of a TCR extracellular domain, and a TCR intracellular domain comprising a stimulatory domain from an intracellular signaling domain of CD3 delta; and a human or humanized antibody domain comprising an antigen binding domain wherein the TCR subunit and the antibody domain are operatively linked, and wherein the TFP incorporates into a TCR when expressed in a T-cell.

[0010] In one aspect, provided herein is an isolated recombinant nucleic acid molecule encoding a T-cell receptor (TCR) fusion protein (TFP) comprising a TCR subunit comprising at least a portion of a TCR

extracellular domain, and a TCR intracellular domain comprising a stimulatory domain from an intracellular signaling domain of TCR alpha; and a human or humanized antibody domain comprising an antigen binding domain wherein the TCR subunit and the antibody domain are operatively linked, and wherein the TFP incorporates into a TCR when expressed in a T-cell.

[0011] In one aspect, provided herein is an isolated recombinant nucleic acid molecule encoding a T-cell receptor (TCR) fusion protein (TFP) comprising a TCR subunit comprising at least a portion of a TCR extracellular domain, and a TCR intracellular domain comprising a stimulatory domain from an intracellular signaling domain of TCR beta; and a human or humanized antibody domain comprising an antigen binding domain wherein the TCR subunit and the antibody domain are operatively linked, and wherein the TFP incorporates into a TCR when expressed in a T-cell.

[0012] In one aspect, provided herein is an isolated recombinant nucleic acid molecule encoding a T-cell receptor (TCR) fusion protein (TFP) comprising a TCR subunit and a human or humanized antibody domain comprising an antigen binding domain that is an anti-mesothelin binding domain.

[0013] In some instances, the TCR subunit and the antibody domain are operatively linked. In some instances, the TFP incorporates into a TCR when expressed in a T-cell. In some instances, the encoded antigen binding domain is connected to the TCR extracellular domain by a linker sequence. In some instances, the encoded linker sequence comprises $(G_4S)_n$, wherein $n=1$ to 4. In some instances, the TCR subunit comprises a TCR extracellular domain. In some instances, the TCR subunit comprises a TCR transmembrane domain. In some instances, the TCR subunit comprises a TCR intracellular domain. In some instances, the TCR subunit comprises (i) a TCR extracellular domain, (ii) a TCR transmembrane domain, and (iii) a TCR intracellular domain, wherein at least two of (i), (ii), and (iii) are from the same TCR subunit. In some instances, the TCR subunit comprises a TCR intracellular domain comprising a stimulatory domain selected from an intracellular signaling domain of CD3 epsilon, CD3 gamma or CD3 delta, or an amino acid sequence having at least one, two or three modifications thereto. In some instances, the TCR subunit comprises an intracellular domain comprising a stimulatory domain selected from a functional signaling domain of 4-1BB and/or a functional signaling domain of CD3 zeta, or an amino acid sequence having at least one modification thereto. In some instances, the human or humanized antibody domain comprises an antibody fragment. In some instances, the human or humanized antibody domain comprises a scFv or a V_H domain. In some instances, the isolated nucleic acid molecule encodes (i) a light chain (LC) CDR1, LC CDR2 and LC CDR3 of an anti-mesothelin light chain binding domain amino acid sequence with 70-100% sequence identity to a light chain (LC) CDR1, LC CDR2 and LC CDR3 of an anti-mesothelin light chain binding domain provided herein, respectively, and/or (ii) a heavy chain (HC) CDR1, HC CDR2 and HC CDR3 of an anti-mesothelin heavy chain binding domain amino acid sequence with 70-100% sequence identity to a heavy chain (HC) CDR1, HC CDR2 and HC CDR3 of an anti-mesothelin heavy chain binding domain provided herein, respectively. In some instances, the isolated nucleic acid molecule encodes a light chain variable region, wherein the light chain variable region comprises an amino acid sequence having at least one but not more than 30 modifications of a light chain variable region amino acid sequence of a light chain variable region

provided herein, or a sequence with 95-99% identity to a light chain variable region amino acid sequence of a light chain variable region provided herein. In some instances, the isolated nucleic acid molecule encodes a heavy chain variable region, wherein the heavy chain variable region comprises an amino acid sequence having at least one but not more than 30 modifications of a heavy chain variable region amino acid sequence of a heavy chain variable region provided herein, or a sequence with 95-99% identity to a heavy chain variable region amino acid sequence of a heavy chain variable region provided herein. In some instances, the TFP includes an extracellular domain of a TCR subunit that comprises an extracellular domain or portion thereof of a protein selected from the group consisting of a TCR alpha chain, a TCR beta chain, a CD3 epsilon TCR subunit, a CD3 gamma TCR subunit, a CD3 delta TCR subunit, functional fragments thereof, and amino acid sequences thereof having at least one but not more than 20 modifications. In some instances, the encoded TFP includes a transmembrane domain that comprises a transmembrane domain of a protein selected from the group consisting of a TCR alpha chain, a TCR beta chain, a CD3 epsilon TCR subunit, a CD3 gamma TCR subunit, a CD3 delta TCR subunit, functional fragments thereof, and amino acid sequences thereof having at least one but not more than 20 modifications. In some instances, the encoded TFP includes a transmembrane domain that comprises a transmembrane domain of a protein selected from the group consisting of a TCR alpha chain, a TCR beta chain, a TCR zeta chain, a CD3 epsilon TCR subunit, a CD3 gamma TCR subunit, a CD3 delta TCR subunit, CD45, CD2, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD28, CD37, CD64, CD80, CD86, CD134, CD137, CD154, functional fragments thereof, and amino acid sequences thereof having at least one but not more than 20 modifications. In some instances, the isolated nucleic acid molecule further comprises a sequence encoding a costimulatory domain. In some instances, the costimulatory domain is a functional signaling domain obtained from a protein selected from the group consisting of DAP10, DAP12, CD30, LIGHT, OX40, CD2, CD27, CD28, CD30, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), and 4-1BB (CD137), and amino acid sequences thereof having at least one but not more than 20 modifications thereto. In some instances, the isolated nucleic acid molecule further comprises a leader sequence. In some instances, the isolated nucleic acid molecule is mRNA.

[0014] In some instances, the TFP includes an immunoreceptor tyrosine-based activation motif (ITAM) of a TCR subunit that comprises an ITAM or portion thereof of a protein selected from the group consisting of CD3 zeta TCR subunit, CD3 epsilon TCR subunit, CD3 gamma TCR subunit, CD3 delta TCR subunit, TCR zeta chain, Fc epsilon receptor 1 chain, Fc epsilon receptor 2 chain, Fc gamma receptor 1 chain, Fc gamma receptor 2a chain, Fc gamma receptor 2b1 chain, Fc gamma receptor 2b2 chain, Fc gamma receptor 3a chain, Fc gamma receptor 3b chain, Fc beta receptor 1 chain, TYROBP (DAP12), CD5, CD16a, CD16b, CD22, CD23, CD32, CD64, CD79a, CD79b, CD89, CD278, CD66d, functional fragments thereof, and amino acid sequences thereof having at least one but not more than 20 modifications thereto. In some instances, the ITAM replaces an ITAM of CD3 gamma, CD3 delta, or CD3 epsilon. In some instances, the ITAM is selected from the group consisting of CD3 zeta TCR subunit, CD3 epsilon TCR subunit, CD3 gamma TCR subunit, and CD3 delta TCR subunit and replaces

a different ITAM selected from the group consisting of CD3 zeta TCR subunit, CD3 epsilon TCR subunit, CD3 gamma TCR subunit, and CD3 delta TCR subunit.

[0015] In some instances, the nucleic acid comprises a nucleotide analog. In some instances, the nucleotide analog is selected from the group consisting of 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), 2'-O-N-methylacetamido (2'-O-NMA) modified, a locked nucleic acid (LNA), an ethylene nucleic acid (ENA), a peptide nucleic acid (PNA), a 1',5'- anhydrohexitol nucleic acid (HNA), a morpholino, a methylphosphonate nucleotide, a thiophosphonate nucleotide, and a 2'-fluoro N3-P5'-phosphoramidite.

[0016] In one aspect, provided herein is an isolated polypeptide molecule encoded by a nucleic acid molecule provided herein.

[0017] In one aspect, provided herein is an isolated TFP molecule comprising a human or humanized anti-mesothelin binding domain, a TCR extracellular domain, a transmembrane domain, and an intracellular domain.

[0018] In one aspect, provided herein is an isolated TFP molecule comprising a human or humanized anti-mesothelin binding domain, a TCR extracellular domain, a transmembrane domain, and an intracellular signaling domain, wherein the TFP molecule is capable of functionally interacting with an endogenous TCR complex and/or at least one endogenous TCR polypeptide.

[0019] In some embodiments, the human or humanized antibody domain comprising an antigen binding domain that is an anti-mesothelin binding domain encoded by the nucleic acid, or an antibody comprising the anti-mesothelin binding domain, or a cell expressing the anti-mesothelin binding domain encoded by the nucleic acid has an affinity value of at most about 200 nM, 100 nM, 75 nM, a 50 nM, 25 nM, 20 nM, 15 nM, 14 nM, 13 nM, 12 nM, 11 nM, 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, 0.9 nM, 0.8 nM, 0.7 nM, 0.6 nM, 0.5 nM, 0.4 nM, 0.3 nM, 0.2 nM, 0.1 nM, 0.09 nM, 0.08 nM, 0.07 nM, 0.06 nM, 0.05 nM, 0.04 nM, 0.03 nM, 0.02 nM, or 0.01 nM; and/or at least about 100 nM, 75 nM, a 50 nM, 25 nM, 20 nM, 15 nM, 14 nM, 13 nM, 12 nM, 11 nM, 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, 0.9 nM, 0.8 nM, 0.7 nM, 0.6 nM, 0.5 nM, 0.4 nM, 0.3 nM, 0.2 nM, 0.1 nM, 0.09 nM, 0.08 nM, 0.07 nM, 0.06 nM, 0.05 nM, 0.04 nM, 0.03 nM, 0.02 nM, or 0.01 nM; and or about 200 nM, 100 nM, 75 nM, a 50 nM, 25 nM, 20 nM, 15 nM, 14 nM, 13 nM, 12 nM, 11 nM, 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, 0.9 nM, 0.8 nM, 0.7 nM, 0.6 nM, 0.5 nM, 0.4 nM, 0.3 nM, 0.2 nM, 0.1 nM, 0.09 nM, 0.08 nM, 0.07 nM, 0.06 nM, 0.05 nM, 0.04 nM, 0.03 nM, 0.02 nM, or 0.01 nM.

[0020] In one aspect, provided herein is an isolated TFP molecule comprising a human or humanized anti-mesothelin binding domain, a TCR extracellular domain, a transmembrane domain, and an intracellular signaling domain, wherein the TFP molecule is capable of functionally integrating into an endogenous TCR complex

[0021] In some instances, the isolated TFP molecule comprises an antibody or antibody fragment comprising a human or humanized anti-mesothelin binding domain, a TCR extracellular domain, a transmembrane domain, and an intracellular domain. In some instances, the anti-mesothelin binding domain is a scFv, a V_H domain, or a camelid V_{HH} domain. In some instances, the anti-mesothelin binding domain comprises a heavy chain with 95-100% identity to an amino acid sequence of a heavy chain provided herein, a functional fragment thereof, or an amino acid sequence thereof having at least one but not more than 30 modifications. In some instances, the anti-mesothelin binding domain comprises a light chain with 95-100% identity to an amino acid sequence of a light chain provided herein, a functional fragment thereof, or an amino acid sequence thereof having at least one but not more than 30 modifications. In some instances, the isolated TFP molecule comprises a TCR extracellular domain that comprises an extracellular domain or portion thereof of a protein selected from the group consisting of a TCR alpha chain, a TCR beta chain, a CD3 epsilon TCR subunit, a CD3 gamma TCR subunit, a CD3 delta TCR subunit, functional fragments thereof, and amino acid sequences thereof having at least one but not more than 20 modifications. In some instances, the anti-mesothelin binding domain is connected to the TCR extracellular domain by a linker sequence. In some instances, the linker region comprises (G₄S)_n, wherein n=1 to 4.

[0022] In some instances, the isolated TFP molecule further comprises a sequence encoding a costimulatory domain. In some instances, the isolated TFP molecule further comprises a sequence encoding an intracellular signaling domain. In some instances, the isolated TFP molecule further comprises a leader sequence.

[0023] In one aspect, provided herein is a vector comprising a nucleic acid molecule encoding a TFP provided herein. In some instances, the vector is selected from the group consisting of a DNA, a RNA, a plasmid, a lentivirus vector, adenoviral vector, a Rous sarcoma viral (RSV) vector, or a retrovirus vector. In some instances, the vector further comprises a promoter. In some instances, the vector is an *in vitro* transcribed vector. In some instances, a nucleic acid sequence in the vector further comprises a poly(A) tail. In some instances, a nucleic acid sequence in the vector further comprises a 3'UTR.

[0024] In one aspect, provided herein is a cell comprising a vector provided herein. In some instances, the cell is a human T-cell. In some instances, the T-cell is a CD8+ or CD4+ T-cell. In some instances, the T cell is a gamma delta T cell. In some instances, the T cell is an NK-T cell. In some instances, the cell further comprises a nucleic acid encoding an inhibitory molecule that comprises a first polypeptide that comprises at least a portion of an inhibitory molecule, associated with a second polypeptide that comprises a positive signal from an intracellular signaling domain. In some instances, the inhibitory molecule comprise first polypeptide that comprises at least a portion of PD1 and a second polypeptide comprising a costimulatory domain and primary signaling domain.

[0024a] In one aspect, provided herein is a human T cell comprising the recombinant nucleic acid molecule as described herein.

[0025] In one aspect, provided herein is a human CD8+ or CD4+ T-cell comprising at least two TFP molecules, the TFP molecules comprising a human or humanized anti-mesothelin binding domain, a

TCR extracellular domain, a transmembrane domain, and an intracellular domain, wherein the TFP molecule is capable of functionally interacting with an endogenous TCR complex and/or at least one endogenous TCR polypeptide in, at and/or on the surface of the human CD8+ or CD4+ T-cell.

[0026] In one aspect, provided herein is a protein complex comprising: a TFP molecule comprising a human or humanized anti-mesothelin binding domain, a TCR extracellular domain, a transmembrane domain, and an intracellular domain; and at least one endogenous TCR complex.

[0027] In some instances, the TCR comprises an extracellular domain or portion thereof of a protein selected from the group consisting of TCR alpha chain, a TCR beta chain, a CD3 epsilon TCR subunit, a CD3 gamma TCR subunit, and a CD3 delta TCR subunit. In some instances, the anti-mesothelin binding domain is connected to the TCR extracellular domain by a linker sequence. In some instances, the linker region comprises (G₄S)_n, wherein n=1 to 4.

[0028] In one aspect, provided herein is a human CD8+ or CD4+ T-cell comprising at least two different TFP proteins per a protein complex provided herein.

[0029] In one aspect, provided herein is a method of making a cell comprising transducing a T-cell with a vector provided herein.

[0030] In one aspect, provided herein is 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 encoding a TFP molecule provided herein.

[0031] In one aspect, provided herein is a method of providing an anti-tumor immunity in a mammal comprising administering to the mammal an effective amount of a cell expressing a TFP molecule provided herein, or expressing a polypeptide molecule provided herein.

[0032] In some instances, the cell is an autologous T-cell. In some instances, the cell is an allogeneic T-cell. In some instances, the mammal is a human.

[0033] In one aspect, provided herein is a method of treating a mammal having a disease associated with expression of mesothelin comprising administering to the mammal an effective amount of a TFP molecule provided herein, a cell provided herein, or a polypeptide molecule provided herein. In some instances, the disease associated with mesothelin expression is selected from the group consisting of a proliferative disease, a cancer, a malignancy, and a non-cancer related indication associated with expression of mesothelin. In some instances, the disease is a cancer selected from the group consisting of mesothelioma, renal cell carcinoma, stomach cancer, breast cancer, lung cancer, ovarian cancer, prostate cancer, colon cancer, cervical cancer, brain cancer, liver cancer, pancreatic cancer, thyroid cancer, bladder cancer, ureter cancer, kidney cancer, endometrial cancer, esophageal cancer, gastric cancer, thymic carcinoma, cholangiocarcinoma and stomach cancer.

[0033a] In one aspect, provided herein is a use of a composition comprising a cell comprising the recombinant nucleic acid molecule as described herein in the manufacture of a medicament for treating a mammal having cancer.

[0033b] In one aspect, provided herein is a composition comprising a cell comprising the recombinant nucleic acid as described herein for use in therapy.

[0034] In some instances, the disease is cancer. In some instances, the disease is selected from the group consisting of mesothelioma, papillary serous ovarian adenocarcinoma, clear cell ovarian carcinoma, mixed Mullerian ovarian carcinoma, endometrioid mucinous ovarian carcinoma, malignang pleural disease, pancreatic adenocarcinoma, ductal pancreatic adenocarcinoma, uterine serous carcinoma, lung adenocarcinoma, extrahepatic bile duct carcinoma, gastric adenocarcinoma, esophageal adenocarcinoma, colorectal adenocarcinoma, breast adenocarcinoma, a disease associated with mesothelin expression, a disease associated with mesothelin expression, non-mucinous ovarian carcinoma, invasive ductal adenocarcinoma, pulmonary adenocarcinoma, gastric/esophageal adenocarcinoma, colorectal adenocarcinoma, leukemia, pediatric acute myeloid leukemia, invasive intraductal papillary mucinous neoplasm (IPMN), endometrial adenocarcinoma, stomach/esophagus adenocarcinoma, pulmonary adenocarcinoma, breast adenocarcinoma, and combinations thereof.

[0035] In some instances, the cells expressing a TFP molecule are administered in combination with an agent that increases the efficacy of a cell expressing a TFP molecule. In some instances, less cytokines are released in the mammal compared a mammal administered an effective amount of a T-cell expressing an anti-mesothelin chimeric antigen receptor (CAR). In some instances, the cells expressing a TFP molecule are administered in combination with an agent that ameliorates one or more side effects

CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154.

[00195] In some instances, the transmembrane domain can be attached to the extracellular region of the TFP, *e.g.*, the antigen binding domain of the TFP, via a hinge, *e.g.*, a hinge from a human protein. For example, in one embodiment, the hinge can be a human immunoglobulin (Ig) hinge, *e.g.*, an IgG4 hinge, or a CD8a hinge.

Linkers

[00196] Optionally, a short oligo- or polypeptide linker, between 2 and 10 amino acids in length may form the linkage between the transmembrane domain and the cytoplasmic region of the TFP. A glycine-serine doublet provides a particularly suitable linker. For example, in one aspect, the linker comprises the amino acid sequence of GGGSGGGGS. In some embodiments, the linker is encoded by a nucleotide sequence of GGTGGCGGAGGTTCTGGAGGTGGAGGTTCC.

Cytoplasmic Domain

[00197] The cytoplasmic domain of the TFP can include an intracellular signaling domain, if the TFP contains CD3 gamma, delta or epsilon polypeptides; TCR alpha and TCR beta subunits are generally lacking in a signaling domain. An intracellular signaling domain is generally responsible for activation of at least one of the normal effector functions of the immune cell in which the TFP has been introduced. The term “effector function” refers to a specialized function of a cell. Effector function of a T-cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines. Thus the term “intracellular signaling domain” refers to the portion of a protein which transduces the effector function signal and directs the cell to perform a specialized function. While usually the entire intracellular signaling domain can be employed, in many cases it is not necessary to use the entire chain. To the extent that a truncated portion of the intracellular signaling domain is used, such truncated portion may be used in place of the intact chain as long as it transduces the effector function signal. The term intracellular signaling domain is thus meant to include any truncated portion of the intracellular signaling domain sufficient to transduce the effector function signal.

[00198] Examples of intracellular signaling domains for use in the TFP of the invention include the cytoplasmic sequences of the T-cell receptor (TCR) and co-receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any recombinant sequence that has the same functional capability.

[00199] It is known that signals generated through the TCR alone are insufficient for full activation of naive T-cells and that a secondary and/or costimulatory signal is required. Thus, naive T-cell activation can be said to be mediated by two distinct classes of cytoplasmic signaling sequences: those that initiate antigen-dependent primary activation through the TCR (primary intracellular signaling domains) and those that act in an antigen-independent manner to provide a secondary or costimulatory signal (secondary cytoplasmic domain, *e.g.*, a costimulatory domain).

[00200] A primary signaling domain regulates primary activation of the TCR complex either in a stimulatory way, or in an inhibitory way. Primary intracellular signaling domains that act in a stimulatory

CLAIMS

WHAT IS CLAIMED IS:

1. A recombinant nucleic acid molecule encoding a T cell receptor (TCR) fusion protein (TFP) comprising
 - (a) a TCR subunit comprising
 - (i) at least a portion of a TCR extracellular domain,
 - (ii) a TCR transmembrane domain, and
 - (iii) a TCR intracellular domain comprising a stimulatory domain from an intracellular signaling domain of CD3 epsilon, CD3 delta or CD3 gamma; and
 - (b) a human or humanized antibody domain comprising an antigen binding domain that is an anti-mesothelin binding domain,wherein the TCR subunit and the human or humanized antibody domain are operatively linked, and
wherein the TFP functionally interacts with a TCR when expressed in a T cell.
2. The recombinant nucleic acid molecule of claim 1, wherein the antigen binding domain is connected to the TCR extracellular domain by a linker sequence.
3. The recombinant nucleic acid molecule of claim 2, wherein the linker sequence comprises $(G_4S)_n$, wherein $n=1$ to 4.
4. The recombinant nucleic acid molecule of claim 1, wherein the TCR subunit comprises (i) a TCR extracellular domain, (ii) a TCR transmembrane domain, and (iii) a TCR intracellular domain, wherein at least two of (i), (ii), and (iii) are from the same TCR subunit.
5. The recombinant nucleic acid molecule of claim 1, wherein the TCR extracellular domain, the TCR transmembrane domain, and the TCR intracellular domain of the TCR subunit are derived from a single subunit, and wherein the single subunit is CD3 epsilon, CD3 gamma or CD3 delta.
6. The recombinant nucleic acid molecule of claim 1, wherein the recombinant nucleic acid molecule is a viral vector.
7. The recombinant nucleic acid molecule of claim 6, wherein the viral vector is a lentiviral vector.
8. The recombinant nucleic acid molecule of claim 1, wherein the anti-mesothelin binding domain

is a V_{HH} domain.

9. The recombinant nucleic acid molecule of claim 1, wherein the anti-mesothelin binding domain comprises a heavy chain (HC) CDR1, a HC CDR2 and a HC CDR3 sequence of SEQ ID NO: 53; or a HC CDR1, a HC CDR2 and a HC CDR3 sequence of SEQ ID NO: 54; wherein the CDR sequences are determined by Kabat numbering.
10. The recombinant nucleic acid molecule of claim 1, wherein the anti-mesothelin binding domain comprises a sequence with 70-100% sequence identity to SEQ ID NO:53 or SEQ ID NO:54.
11. The recombinant nucleic acid molecule of claim 1, wherein the anti-mesothelin binding domain comprises the sequence of SEQ ID NO:53 or SEQ ID NO:54.
12. The recombinant nucleic acid molecule of claim 1, wherein the TCR subunit comprises SEQ ID NO: 4, SEQ ID NO: 5, or SEQ ID NO: 6.
13. A human T cell comprising the recombinant nucleic acid molecule of claim 1.
14. The human T cell of claim 13, wherein the human T cell exhibits increased cytotoxicity to a cell expressing mesothelin compared to a T cell not containing the TFP.
15. The human T cell of claim 13, wherein production of IL-2 or IFN γ by the human T cell is increased in the presence of a cell expressing mesothelin.
16. The human T cell of claim 13, wherein the human T cell has greater than or more efficient cytotoxic activity than a CD8⁺ or CD4⁺ T cell comprising a nucleic acid encoding a chimeric antigen receptor (CAR) comprising (a) the anti-mesothelin binding domain, operatively linked to (b) at least a portion of a CD28 extracellular domain, (c) a CD28 transmembrane domain, (d) at least a portion of a CD28 intracellular domain, and (e) a CD3 zeta intracellular domain.
17. The human T cell of claim 13, wherein the human T cell is a CD8⁺ T cell, a CD4⁺ T cell, a CD4⁺ CD8⁺ T cell, a gamma-delta T cell, an alpha-beta T cell or a NKT cell.
18. Use of a composition comprising a cell comprising the recombinant nucleic acid molecule of any one of claims 1-12 in the manufacture of a medicament for treating a mammal having cancer.
19. The use of claim 18, wherein the cancer is selected from the group consisting of mesothelioma, renal cell carcinoma, stomach cancer, breast cancer, lung cancer, ovarian cancer, prostate cancer, colon cancer, cervical cancer, brain cancer, liver cancer, pancreatic cancer, thyroid cancer, bladder cancer, ureter cancer, kidney cancer, endometrial cancer, esophageal cancer,

gastric cancer, thymic carcinoma, cholangiocarcinoma, and metastases thereof.

20. A composition comprising a cell comprising the recombinant nucleic acid of any one of claims 1-12 for use in therapy.