

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) a TCR extracellular domain, (ii) a TCR transmembrane domain, and (iii) a TCR intracellular domain, 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 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 antigen binding domain is operatively linked to N-terminus of the TCR extracellular domain, 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

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 $(G_4S)_n$, 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 molecule as described herein for use in therapy.

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) a TCR extracellular domain,
 - (ii) a TCR transmembrane domain, and
 - (iii) a TCR intracellular domain,
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 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 antigen binding domain is operatively linked to N-terminus of the TCR extracellular domain, 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 single subunit is CD3 epsilon.
5. The recombinant nucleic acid molecule of claim 1, wherein the recombinant nucleic acid molecule is a viral vector.
6. The recombinant nucleic acid molecule of claim 5, wherein the viral vector is a lentiviral vector.
7. The recombinant nucleic acid molecule of claim 1, wherein the anti-mesothelin binding domain is a V_{HH} domain.
8. A human T cell comprising the recombinant nucleic acid molecule of claim 1.

9. The human T cell of claim 8, wherein the human T cell exhibits increased cytotoxicity to a cell expressing mesothelin compared to a T cell not containing the TFP.
10. The human T cell of claim 8, wherein production of IL-2 or IFN γ by the human T cell is increased in the presence of a cell expressing mesothelin.
11. The human T cell of claim 8, 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.
12. The human T cell of claim 8, 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.
13. Use of a composition comprising a cell comprising the recombinant nucleic acid molecule of any one of claims 1-7 in the manufacture of a medicament for treating a mammal having cancer.
14. The use of claim 13, 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.
15. A composition comprising a cell comprising the recombinant nucleic acid molecule of any one of claims 1-7 for use in therapy.