

expansion, persistence over time, and, in case of relapsing disease, to enable a ‘memory’ response. High and manageable clinical efficacy of CAR T cells is currently limited to CD19-positive B cell malignancies and to NY-ESO-1-peptide expressing synovial sarcoma patients expressing HLA-A2.

SUMMARY OF THE INVENTION

[0005] There is a clear need to improve genetically engineered T cells to more broadly act against various human malignancies.

[0006] Described herein are modified T cells comprising fusion proteins of TCR subunits, including CD3 epsilon, CD3 gamma, CD3 delta, TCR gamma, TCR delta, TCR alpha and TCR beta chains with binding domains specific for cell surface antigens that have the potential to overcome limitations of existing approaches. Additionally, these modified T cells may have functional disruption of an endogenous TCR (e.g. TCR alpha, beta or both). These modified T cells may have the ability to kill target cells more efficiently than CARs, but release comparable or lower levels of pro-inflammatory cytokines. These modified T cells and methods of their use may represent an advantage for these cells relative to CARs because elevated levels of these cytokines have been associated with dose-limiting toxicities for adoptive CAR-T therapies.

[0007] Provided herein are modified T cells comprising T-cell receptor (TCR) fusion protein (TFP) and a TCR constant domain, methods of producing the modified T cells, and methods of use thereof for the treatment of diseases.

[0008] Disclosed herein, in some embodiments, are recombinant nucleic acid comprising (a) a sequence encoding a T cell receptor (TCR) fusion protein (TFP) comprising (i) a TCR subunit comprising (1) at least a portion of a TCR extracellular domain, (2) a transmembrane domain, and (3) an intracellular domain comprising a stimulatory domain from an intracellular signaling domain of CD3 epsilon, CD3 gamma, CD3 delta, TCR gamma, TCR delta, TCR alpha or TCR beta, and (ii) a human or humanized antibody comprising an antigen binding domain; and (b) a sequence encoding a TCR constant domain, wherein the TCR constant domain is a TCR alpha constant domain, a TCR beta constant domain, a TCR gamma constant domain, a TCR delta constant domain, or a TCR gamma constant domain and a TCR delta constant domain; wherein the TCR subunit and the antibody are operatively linked, and wherein the TFP functionally incorporates into a TCR complex when expressed in a T cell.

[0008a] Disclosed herein, in some embodiments, are recombinant nucleic acid comprising

- (a) a sequence encoding a T cell receptor (TCR) fusion protein (TFP) comprising
 - (i) a TCR subunit comprising
 - (1) at least a portion of a TCR extracellular domain,
 - (2) a transmembrane domain, and
 - (3) an intracellular domain comprising a stimulatory domain from an intracellular signaling domain of CD3 epsilon, CD3 gamma, CD3 delta, TCR alpha or TCR beta, and
 - (ii) an antibody comprising an antigen binding domain; and
- (b) a sequence encoding a TCR constant domain, wherein the TCR constant domain is a TCR alpha constant domain, a TCR beta constant domain or a TCR alpha constant domain and a TCR beta constant domain;

wherein the TCR subunit and the antibody are operatively linked, and

wherein the TFP functionally incorporates into a TCR complex when expressed in a modified T cell comprising a functional disruption of an endogenous TCR.

[0016a] Disclosed herein, in some embodiments, are use of the pharmaceutical composition of as described herein in the manufacture of a medicament for treating cancer in a subject in need thereof.

[0016b] Disclosed herein, in some embodiments, are the pharmaceutical composition as described herein for use in therapy.

[0017] Disclosed herein, in some embodiments, are recombinant nucleic acid, the vector, the modified T cell, or the pharmaceutical composition disclosed herein, for use as a medicament or in the preparation of a medicament.

INCORPORATION BY REFERENCE

[0018] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] **FIG. 1** is a sequence alignment between TRBC1 and TRBC2, selected crRNAs are represented by arrows over TRBC1 sequence.

[0020] **FIGs. 2A-B** depict example graphs showing surface expression of CD3 (SK7) vs TCR $\alpha\beta$ (IP26) in TRA-edited (**FIG. 2A**) and TRB-edited (**FIG. 2B**) cells. Wild type Jurkat cells were edited at either the TRAC or TRBC genes to disrupt TRA or TRB surface expression. Cells negative for CD3 and TCR $\alpha\beta$ were purified using Magnetic-Activated Cell Sorting. The gates on the plots were drawn to delineate CD3 and TCR $\alpha\beta$ negative-negative population of cells and the percentages of cells remaining in each quadrant are shown in the corners.

[0021] **FIGs. 3A-E** depict example graphs showing surface expression of CD3 vs TCR $\alpha\beta$ in wild type cells vs edited (TRA/B disrupted) cells before and after purification. Wild type Donor 1 T cells (**FIG. 3A**) were edited at either the TRAC (**FIG. 3B** or **FIG. 3D**) or TRBC (**FIG. 3C** or **FIG. 3E**) genes to disrupt TRA or TRB surface expression. **FIG. 3B** and **FIG. 3C** show status of CD3 vs TCR $\alpha\beta$ surface markers directly after editing, while **FIG. 3D** and **FIG. 3E** show status of these surface markers after their negative selection using Magnetic-Activated Cell Sorting (MACS). The gates on the plots were drawn to delineate CD3 and TCR $\alpha\beta$ negative-negative population of cells and the percentages of cells remaining in each quadrant are shown in the corners.

[0022] FIG. 4 depicts example graphs measuring the allogenicity of TCR-negative T cells by observing their proliferation rates. TCR-negative T cells were permanently labelled with CFSE dye which halves its concentration with cellular division. Full gray peaks along the X-axis show CFSE signal in unlabeled cells as a negative control. Gray lines show CFSE amount in cells after 24 hours without any stimulation while black lines indicate CFSE

CLAIMS

WHAT IS CLAIMED IS:

1. A recombinant nucleic acid comprising
 - (a) a sequence encoding a T cell receptor (TCR) fusion protein (TFP) comprising
 - (i) a TCR subunit comprising
 - (1) at least a portion of a TCR extracellular domain,
 - (2) a transmembrane domain, and
 - (3) an intracellular domain comprising a stimulatory domain from an intracellular signaling domain of CD3 epsilon, CD3 gamma, CD3 delta, TCR alpha or TCR beta, and
 - (ii) an antibody comprising an antigen binding domain; and
 - (b) a sequence encoding a TCR constant domain, wherein the TCR constant domain is a TCR alpha constant domain, a TCR beta constant domain or a TCR alpha constant domain and a TCR beta constant domain;wherein the TCR subunit and the antibody are operatively linked, and
wherein the TFP functionally incorporates into a TCR complex when expressed in a modified T cell comprising a functional disruption of an endogenous TCR.
2. The recombinant nucleic acid of claim 1, wherein the TCR subunit and the antibody domain are operatively linked by a linker sequence; optionally wherein the linker sequence comprises (G4S)_n, wherein n=1 to 4.
3. The recombinant nucleic acid of claim 1 or 2, wherein the transmembrane domain is a TCR transmembrane domain from CD3 epsilon, CD3 gamma, CD3 delta, TCR alpha or TCR beta;
wherein the intracellular domain is derived from only CD3 epsilon, only CD3 gamma, only CD3 delta, only TCR alpha or only TCR beta; and/or
wherein the TCR extracellular domain 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.
4. The recombinant nucleic acid of any one of claims 1-3, wherein the TCR subunit comprises (i) at least a portion of 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 of any one of claims 1-4, wherein

- (a) the TCR constant domain is a TCR alpha constant domain and the TFP functionally integrates into a TCR complex comprising an endogenous subunit of TCR beta, CD3 epsilon, CD3 gamma, CD3 delta, or a combination thereof;
- (b) the TCR constant domain is a TCR beta constant domain and the TFP functionally integrates into a TCR complex comprising an endogenous subunit of TCR alpha, CD3 epsilon, CD3 gamma, CD3 delta, or a combination thereof; or
- (c) the TCR constant domain is a TCR alpha constant domain and a TCR beta constant domain and the TFP functionally integrates into a TCR complex comprising an endogenous subunit of CD3 epsilon, CD3 gamma, CD3 delta, or a combination thereof.

6. The recombinant nucleic acid of any one of claims 1-5, wherein the antibody is an antibody fragment; optionally wherein the antibody fragment is a scFv, a single domain antibody domain, a VH domain or a VL domain.

7. The recombinant nucleic acid of any one of claims 1-6, wherein an antigen binding domain is selected from a group consisting of an anti-CD19 binding domain, anti-B-cell maturation antigen (BCMA) binding domain, anti-mesothelin (MSLN) binding domain, an anti-IL13R α 2 binding domain, an anti-MUC16 binding domain, an anti-CD22 binding domain, an anti-PD-1 binding domain, an anti BAFF or BAFF receptor binding domain, and anti-ROR-1 binding domain.

8. The recombinant nucleic acid of any one of claims 1-7, further comprising:

- (a) a leader sequence;
- (b) a promoter sequence;
- (c) a sequence encoding a poly(A) tail;
- (d) a 3'UTR sequence;
- (e) a sequence encoding a TCR alpha transmembrane domain;
- (f) a sequence encoding a TCR beta transmembrane domain; and/or
- (g) a sequence encoding a TCR alpha transmembrane domain and a sequence encoding a TCR beta transmembrane domain.

9. A vector comprising the recombinant nucleic acid of any one of claims 1-8.

10. A modified T cell comprising the recombinant nucleic acid of any one of claims 1-8, or the vector of claim 9, wherein the modified T cell comprises a functional disruption of an endogenous TCR.

11. The modified T cell of claim 10, wherein the T cell further comprises a heterologous sequence encoding a TCR constant domain, wherein the TCR constant domain is a TCR alpha constant domain, a TCR beta constant domain or a TCR alpha constant domain and a TCR beta constant domain.

12. The modified T cell of claim 10 or 11, wherein the endogenous TCR that is functionally disrupted is an endogenous TCR alpha chain, an endogenous TCR beta chain, or an endogenous TCR alpha chain and an endogenous TCR beta chain;

wherein the endogenous TCR that is functionally disrupted has reduced binding to MHC-peptide complex compared to that of an unmodified control T cell; and/or

wherein the functional disruption is a disruption of a gene encoding the endogenous TCR, optionally wherein the disruption of a gene encoding the endogenous TCR is a removal of a sequence of the gene encoding the endogenous TCR from the genome of a T cell.

13. The modified T cell of any one of claims 10-12, further comprising a nucleic acid encoding an inhibitory molecule that comprises a first polypeptide comprising at least a portion of an inhibitory molecule, associated with a second polypeptide comprising a positive signal from an intracellular signaling domain;

optionally wherein the inhibitory molecule comprises the first polypeptide comprising at least a portion of PD1 and the second polypeptide comprising a costimulatory domain and primary signaling domain.

14. A pharmaceutical composition comprising:

- (a) the modified T cells of any one of claims 10-13; and
- (b) a pharmaceutically acceptable carrier.

15. A method of producing the modified T cell of any one of claims 10-13, the method comprising:

- (a) disrupting an endogenous TCR gene encoding a TCR alpha chain, a TCR beta chain, or a TCR alpha chain and a TCR beta chain; thereby producing a T cell containing a functional disruption of an endogenous TCR gene; and
- (b) transducing the T cell containing a functional disruption of an endogenous TCR gene with the recombinant nucleic acid of any one of claims 1-7, or the vector of claim 8.

16. The method of claim 15, wherein disrupting comprises transducing the T cell with a nuclease protein or a nucleic acid sequence encoding a nuclease protein that targets the endogenous gene encoding a TCR alpha chain, a TCR beta chain, or a TCR alpha chain and a TCR beta chain;

optionally wherein the nuclease is a meganuclease, a zinc-finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN), a CRISPR/Cas nuclease, or a megaTAL nuclease.

17. The method of claim 15 or 16, wherein the sequence comprised by the recombinant nucleic acid or the vector is inserted into the endogenous TCR subunit gene at the cleavage site, and wherein the insertion of the sequence into the endogenous TCR subunit gene functionally disrupts the endogenous TCR subunit.

18. Use of the pharmaceutical composition of claim 14 in the manufacture of a medicament for treating cancer in a subject in need thereof.

19. The use of claim 18, wherein the cancer is a solid cancer, a lymphoma or a leukemia; optionally wherein the cancer is selected from the group consisting of renal cell carcinoma, breast cancer, lung cancer, ovarian cancer, prostate cancer, colon cancer, cervical cancer, brain cancer, liver cancer, pancreatic cancer, kidney and stomach cancer.

20. The pharmaceutical composition of claim 14 for use in therapy.