

CLAIMS

We claim:

1. A modified T cell comprising:

a functional exogenous receptor comprising:

(a) an extracellular ligand binding domain,

(b) a transmembrane domain, and

(c) an intracellular signaling domain (“ISD”) comprising a chimeric signaling domain (“CMSD”),

wherein the CMSD comprises one or a plurality of Immune-receptor Tyrosine-based Activation Motifs (“CMSD ITAMs”), wherein the plurality of CMSD ITAMs are optionally connected by one or more linkers (“CMSD linkers”).

2. The modified T cell of claim 1, wherein:

(a) the plurality of CMSD ITAMs are directly linked to each other;

(b) the CMSD comprises two or more CMSD ITAMs connected by one or more CMSD linkers not derived from an ITAM-containing parent molecule;

(c) the CMSD comprises one or more CMSD linkers derived from an ITAM-containing parent molecule that is different from the ITAM-containing parent molecule from which one or more of the CMSD ITAMs are derived from;

(d) the CMSD comprises two or more identical CMSD ITAMs;

(e) at least one of the CMSD ITAMs is not derived from CD3 ζ ;

(f) at least one of the CMSD ITAMs is not ITAM1 or ITAM2 of CD3 ζ ;

(g) the plurality of CMSD ITAMs are each derived from a different ITAM-containing parent molecule; and/or

(h) at least one of the CMSD ITAMs is derived from an ITAM-containing parent molecule selected from the group consisting of CD3 ϵ , CD3 δ , CD3 γ , Ig α (CD79a), Ig β (CD79b), Fc ϵ RI β , Fc ϵ RI γ , DAP12, CNAIP/NFAM1, STAM-1, STAM-2, and Moesin.

~~3. The modified T cell of claim 1, wherein at least one of the CMSD ITAMs is derived from an ITAM-containing parent molecule selected from the group consisting of CD3 ϵ , CD3 δ , CD3 γ , CD3 ζ , Ig α (CD79a), Ig β (CD79b), Fc ϵ RI β , Fc ϵ RI γ , DAP12, CNAIP/NFAM1, STAM-1, STAM-2, and Moesin.~~

43. The modified T cell of any one of preceding claims 1-3, wherein:

i) the CMSD does not comprise ITAM1 and/or ITAM2 of CD3 ζ ;

- ~~5. The modified T cell of any one of claims 1-4, wherein ii) the CMSD comprises ITAM3 of CD3 ζ ;~~
- ~~6. The modified T cell of any one of claims 1-5, wherein iii) the CMSD comprises at least two of the CMSD ITAMs, wherein the at least two CMSD ITAMs: (a) are derived from the same ITAM-containing parent molecule;~~
- ~~7. The modified T cell of claim 6, optionally wherein the at least two of the CMSD ITAMs are identical to each other;~~
- ~~8. The modified T cell of any one of claims 1-6, wherein at least two of the CMSD ITAMs or are different ~~from~~to each other;~~
- ~~9. The modified T cell of claim 8, wherein the two; or (b) are different CMSD ITAMs from each other and are each derived from a different ITAM-containing parent molecule;~~
- ~~10. The modified T cell of any one of claims 1-9, wherein iv) at least one of the CMSD linkers is derived from CD3 ζ ;~~
- ~~11. The modified T cell of any one of claims 1-10, wherein v) at least one of the CMSD linkers is heterologous to the ITAM-containing parent molecule;~~
- ~~12. The modified T cell of any one of claims 1-11, wherein vi) the CMSD further comprises a C-terminal sequence at the C-terminus of the most C-terminal CMSD ITAM (“(CMSD C-terminal sequence)”);~~
- ~~13. The modified T cell of any one of claims 1-12, vii) wherein the CMSD further comprises an N-terminal sequence at the N-terminus of the most N-terminal CMSD ITAM (“(CMSD N-terminal sequence)”); and/or~~
- ~~14. The modified T cell of any one of claims 1-13, viii) wherein the one or more CMSD linkers, the CMSD C-terminal sequence, and/or the CMSD N-terminal sequence are independently selected from the group consisting of SEQ ID NOs: 17-39 and 116-120.~~
- ~~154. The modified T cell of any ~~one of~~preceding claims 1-14, wherein the CMSD comprising a sequence selected from the group consisting of SEQ ID NOs: 41-74.~~
5. The modified T cell of any preceding claims, wherein the functional exogenous receptor is an ITAM-modified T cell receptor (TCR), an ITAM-modified chimeric antigen receptor (CAR), an ITAM-modified chimeric TCR (cTCR), or an ITAM-modified T cell antigen coupler (TAC)-like chimeric receptor; optionally wherein:
- ~~16. The modified T cell of claim 15, wherein the functional exogenous receptor is an ITAM-modified CAR;~~

- ~~17. The modified T cell of claim 16, further optionally wherein the transmembrane domain is derived from CD8 α .~~
- ~~18. The modified T cell of claim 16 or 17, wherein the; and/or the ISD further comprises a co-stimulatory signaling domain; optionally wherein:~~
- ~~19. The modified T cell of claim 18, wherein a) the co-stimulatory signaling domain is derived from CD137 (4-1BB) or CD28;~~
- ~~20. The modified T cell of claim 18 or 19, wherein b) the co-stimulatory signaling domain comprises the amino acid sequence of SEQ ID NO: 124; and/or~~
- ~~21. The modified T cell of any one of claims 18-20, wherein c) the co-stimulatory domain is N-terminal to the CMSD.~~
- ~~22. The modified T cell of any one of claims 18-20, wherein the co-stimulatory domain is, or C-terminal to the CMSD.~~
- ~~236. The modified T cell of claim 155, wherein the ITAM-modified CAR comprising the sequence of any of SEQ ID NOs: 76-96, 98-104 and 106-113.~~
7. The modified T cell of claim 5, wherein the functional exogenous receptor is an ITAM-modified cTCR; further optionally wherein:
- ~~24. The modified T cell of claim 23, wherein the ITAM-modified cTCR comprises:~~
- (a) an extracellular ligand binding domain,
 - (b) an optional receptor domain linker,
 - (c) an optional extracellular domain of a first TCR subunit or a portion thereof,
 - (d) a transmembrane domain comprising a transmembrane domain of a second TCR subunit, and
 - (e) an ISD comprising the CMSD,
- wherein the first and second TCR subunits are selected from the group consisting of TCR α , TCR β , TCR γ , TCR δ , CD3 ϵ , CD3 γ , and CD3 δ ; optionally.
- ~~25. The modified T cell of claim 24, wherein the first and second TCR subunits are both CD3 ϵ .~~
- ~~26. The modified T cell of claim 24; and/or-25,~~
- wherein the one or plurality of CMSD ITAMs are derived from one or more of CD3 ϵ , CD3 δ , and CD3 γ .
- ~~278. The modified T cell of claim 155, wherein the functional exogenous receptor is an ITAM-modified TAC-like chimeric receptor.~~
- ~~28. The modified T cell of claim 27; further optionally wherein:~~
- the ITAM-modified TAC-like chimeric receptor comprises:

- (a) an extracellular ligand binding domain,
- (b) an optional first receptor domain linker,
- (c) an extracellular TCR binding domain that specifically recognizes the extracellular domain of a first TCR subunit,
- (d) an optional second receptor domain linker,
- (e) an optional extracellular domain of a second TCR subunit or a portion thereof,
- (f) a transmembrane domain comprising a transmembrane domain of a third TCR subunit, and
- (g) an ISD comprising the CMSD,

wherein the first, second, and third TCR subunits are all selected from the group consisting of TCR α , TCR β , TCR γ , TCR δ , CD3 ϵ , CD3 γ , and CD3 δ .~~_____~~; optionally:

~~29. The modified T cell of claim 28, wherein the second and third TCR subunits are both CD3 ϵ ;~~

~~30. The modified T cell of claim 28; and/or 29,~~

wherein the one or plurality of CMSD ITAMs are derived from one or more of CD3 ϵ , CD3 δ , and CD3 γ .~~_____~~

~~31.~~ The modified T cell of any ~~one of~~ preceding claims ~~1-30~~, wherein:

i) the extracellular ligand binding domain comprises one or more antigen-binding fragments that specifically recognizing one or more epitopes of one or more target antigens;~~;~~

~~32. The modified T cell of claim 31, optionally a)~~ wherein the antigen-binding fragment is an sdAb or an scFv;

~~33. The modified T cell of claim 31; and/or 32, b)~~ wherein the target antigen is BCMA, CD19, or CD20;~~;~~

~~34. The ii) the modified T cell of any one of claims 1-33, further comprising~~ comprises a hinge domain located between the C-terminus of the extracellular ligand binding domain and the N-terminus of the transmembrane domain;~~;~~

~~35. The modified T cell of claim 34, optionally~~ wherein the hinge domain is derived from CD8 α ;~~;~~

~~36. The modified T cell of any one of claims 1-35, wherein iii)~~ the effector function of the functional exogenous receptor comprising the ISD that comprises the CMSD is at most about 80% less than a functional exogenous receptor comprising an ISD that comprises an intracellular signaling domain of CD3 ζ .

~~37~~10. A method of producing a modified T cell, comprising introducing into a precursor T cell a nucleic acid encoding a functional exogenous receptor,

wherein the functional exogenous receptor comprises:

- (a) an extracellular ligand binding domain,
- (b) a transmembrane domain, and
- (c) an ISD comprising a CMSD,

wherein the CMSD comprises one or a plurality of CMSD ITAMs, wherein the plurality of CMSD ITAMs are optionally connected by one or more CMSD linkers.

~~38~~11. The method of claim ~~37~~10, wherein:

i) the nucleic acid is on a viral vector;

~~39. The~~i) the method of claim ~~37 or 38~~, further ~~comprising~~comprises isolating and/or enriching functional exogenous receptor-positive T cells from the modified T cells.

~~40. The method of any one of claims 37-39, further comprising; and/or~~ formulating the modified T cell with at least one pharmaceutically acceptable carrier.

~~41~~12. The method of ~~any one of claims 37-40~~claim 10 or 11, wherein:

- (a) the plurality of CMSD ITAMs are directly linked to each other;
- (b) the CMSD comprises two or more CMSD ITAMs connected by one or more CMSD linkers not derived from an ITAM-containing parent molecule;
- (c) the CMSD comprises one or more CMSD linkers derived from an ITAM-containing parent molecule that is different from the ITAM-containing parent molecule from which one or more of the CMSD ITAMs are derived from;
- (d) the CMSD comprises two or more identical CMSD ITAMs;
- (e) at least one of the CMSD ITAMs is not derived from CD3 ζ ;
- (f) at least one of the CMSD ITAMs is not ITAM1 or ITAM2 of CD3 ζ ;
- (g) the plurality of CMSD ITAMs are each derived from a different ITAM-containing parent molecule; and/or

(h) at least one of the CMSD ITAMs is derived from an ITAM-containing parent molecule selected from the group consisting of CD3 ϵ , CD3 δ , CD3 γ , Ig α (CD79a), Ig β (CD79b), Fc ϵ RI β , Fc ϵ RI γ , DAP12, CNAIP/NFAM1, STAM-1, STAM-2, and Moesin.

~~42. The method of any one of claims 37-41, wherein at least one of the CMSD ITAMs is derived from an ITAM-containing parent molecule selected from the group consisting of CD3 ϵ , CD3 δ , CD3 γ , CD3 ζ , Ig α (CD79a), Ig β (CD79b), Fc ϵ RI β , Fc ϵ RI γ , DAP12, CNAIP/NFAM1, STAM-1, STAM-2, and Moesin.~~

~~43~~13. A modified T cell obtained by the method of any one of claims ~~37-42~~10-12.

~~44~~14. A pharmaceutical composition comprising the modified T cell of any one of claims ~~1-36~~9 and ~~43~~13, and a pharmaceutically acceptable carrier.

~~4515. A method of treating a disease in an individual, comprising administering to the individual~~
Use of an effective amount of the modified T cell of any one of claims 1-369 and 4313, or the pharmaceutical composition of claim 4414, in the manufacture of a medicament for treating a disease in an individual.

~~4616. The method~~use of claim 4515, wherein

i) the disease is cancer;

~~47. The method of claim 45 or 46, wherein~~ii) the individual is histoincompatible with the donor of the precursor T cell from which the modified T cell is derived; and/or

~~48. The method of any one of claims 45-47, wherein~~iii) the individual is a human.

~~4917. An isolated nucleic acid encoding a functional exogenous receptor,~~
wherein the functional exogenous receptor comprises:

(a) an extracellular ligand binding domain,

(b) a transmembrane domain, and

(c) an ISD comprising a CMSD,

wherein the CMSD comprises one or a plurality of CMSD ITAMs, wherein the plurality of CMSD ITAMs are optionally connected by one or more CMSD linkers.

~~5018. The isolated nucleic acid of claim 4917, wherein at least one of the CMSD ITAMs is derived from an ITAM-containing parent molecule selected from the group consisting of~~
CD3 ϵ , CD3 δ , CD3 γ , CD3 ζ , Ig α (CD79a), Ig β (CD79b), Fc ϵ RI β , Fc ϵ RI γ , DAP12, CNAIP/NFAM1, STAM-1, STAM-2, and Moesin.

~~5119. A vector comprising the nucleic acid of claim 4917 or 5018,~~

~~52. The optionally wherein the vector of claim 51, which is a viral vector.~~