

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 any preceding claims, wherein:
 - i) the CMSD does not comprise ITAM1 and/or ITAM2 of CD3 ζ ;
 - ii) the CMSD comprises ITAM3 of CD3 ζ ;
 - iii) the CMSD comprises at least two CMSD ITAMs, wherein the at least two CMSD ITAMs: (a) are derived from the same ITAM-containing parent molecule, optionally wherein the at least two of the CMSD ITAMs are identical to each other or are different to each other;

or (b) are different from each other and are each derived from a different ITAM-containing parent molecule;

iv) at least one of the CMSD linkers is derived from CD3 ζ ;

v) at least one of the CMSD linkers is heterologous to the ITAM-containing parent molecule;

vi) the CMSD further comprises a C-terminal sequence at the C-terminus of the most C-terminal CMSD ITAM (CMSD C-terminal sequence);

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

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.

4. The modified T cell of any preceding claims, 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:
the functional exogenous receptor is an ITAM-modified CAR, further optionally wherein the transmembrane domain is derived from CD8 α ; and/or the ISD further comprises a co-stimulatory signaling domain; optionally wherein:

- a) the co-stimulatory signaling domain is derived from CD137 (4-1BB) or CD28;
- b) the co-stimulatory signaling domain comprises the amino acid sequence of SEQ ID NO: 124; and/or
- c) the co-stimulatory domain is N-terminal to the CMSD, or C-terminal to the CMSD.

6. The modified T cell of claim 5, 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:

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,
wherein the first and second TCR subunits are both CD3 ϵ ; and/or
wherein the one or plurality of CMSD ITAMs are derived from one or more of CD3 ϵ , CD3 δ , and CD3 γ .

8. The modified T cell of claim 5, wherein the functional exogenous receptor is an ITAM-modified TAC-like chimeric receptor; 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:
wherein the second and third TCR subunits are both CD3 ϵ ; and/or
wherein the one or plurality of CMSD ITAMs are derived from one or more of CD3 ϵ , CD3 δ , and CD3 γ .

9. The modified T cell of any preceding claims, 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;
optionally a) wherein the antigen-binding fragment is an sdAb or an scFv; and/or b) wherein the target antigen is BCMA, CD19, or CD20;
ii) the modified T cell further comprises a hinge domain located between the C-terminus of the extracellular ligand binding domain and the N-terminus of the transmembrane domain;
optionally wherein the hinge domain is derived from CD8 α ;
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 .

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.
11. The method of claim 10, wherein:
- i) the nucleic acid is on a viral vector;
 - ii) the method further comprises isolating and/or enriching functional exogenous receptor-positive T cells from the modified T cells; and/or formulating the modified T cell with at least one pharmaceutically acceptable carrier.
12. The method of 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.
13. A modified T cell obtained by the method of any one of claims 10-12.
14. A pharmaceutical composition comprising the modified T cell of any one of claims 1-9 and 13, and a pharmaceutically acceptable carrier.
15. Use of an effective amount of the modified T cell of any one of claims 1-9 and 13, or the pharmaceutical composition of claim 14, in the manufacture of a medicament for treating a disease in an individual.

16. The use of claim 15, wherein
- i) the disease is cancer;
 - ii) the individual is histoincompatible with the donor of the precursor T cell from which the modified T cell is derived; and/or
 - iii) the individual is a human.
17. 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.
18. The isolated nucleic acid of claim 17, 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.
19. A vector comprising the nucleic acid of claim 17 or 18, optionally wherein the vector is a viral vector.