

CLAIMS

What is claimed is:

1. A chimeric fusion receptor (CFR), wherein the CFR comprises an ectodomain, a transmembrane domain, and an endodomain, and wherein the ectodomain, the transmembrane domain and the endodomain do not comprise any endoplasmic reticulum (ER) retention signals or endocytosis signals.
2. The chimeric fusion receptor of claim 1, wherein the ectodomain is not an scFv (single-chain variable fragment) of an antibody; wherein the ectodomain initiates signal transduction upon binding to a selected agonist; wherein the endodomain comprises at least one signaling domain that activates a selected signaling pathway for enhancing cell therapeutic properties; wherein the CFR is cell surface presented when expressed; and wherein the CFR has reduced internalization and surface downregulation.
3. The chimeric fusion receptor of claim 1, wherein the endodomain and the ectodomain are modular; or wherein for a given endodomain of the CFR, the ectodomain is switchable depending on binding specificity of a selected agonist; or wherein for a given ectodomain, the endodomain is switchable depending on a selected signaling pathway for regulation.
4. The chimeric fusion receptor of claim 1 or 2, wherein the ectodomain comprises a full or partial length of an extracellular portion of a signaling protein comprising at least one of CD3 ϵ , CD3 γ , CD3 δ , CD28, CD5, CD16, CD64, CD32, CD33, CD89, NKG2C, NKG2D, any functional variants, and a combination or a chimera thereof.
5. The chimeric fusion receptor of claim 2, wherein the selected agonist is an agonistic ligand comprising (i) an antibody or a functional variant or fragment thereof; or (ii) an engager; and wherein the selected agonist comprises at least a binding domain specific to a portion of the ectodomain of the CFR.
6. The chimeric fusion receptor of claim 5, wherein the selected agonist comprises at least a binding domain that is specific to an extracellular portion of CD3 ϵ , CD3 γ , CD3 δ , CD28, CD5, CD16, CD64, CD32, CD33, CD89, NKG2C, NKG2D, or any functional variants thereof; or

wherein the selected agonist is an engager that further comprises a binding domain specific to at least one tumor antigen comprising B7H3, BCMA, CD10, CD19, CD20, CD22, CD24, CD30, CD33, CD34, CD38, CD44, CD79a, CD79b, CD123, CD138, CD179b, CEA, CLEC12A, CS-1, DLL3, EGFR, EGFRvIII, EpCAM, FLT-3, FOLR1, FOLR3, GD2, gpA33, HER2, HM1.24, LGR5, MSLN, MCSP, MICA/B, PSMA, PAMA, P-cadherin, or ROR1.

7. The chimeric fusion receptor of claim 5, wherein:

(i) the ectodomain comprises a full or partial length of an extracellular portion of: (a) CD3 ϵ , CD3 γ , CD3 δ , any functional variants, or combinational or chimeric forms thereof; (b) a heterodimer of CD3 ϵ and CD3 γ ; or (c) a heterodimer of CD3 ϵ and CD3 δ ; and

(ii) the selected agonist has a binding specificity to the ectodomain of CD3; or wherein the selected agonist comprises at least one of CD3 $\times$ CD19, CD3 $\times$ CD20, CD3 $\times$ CD33, blinatumomab, catumaxomab, ertumaxomab, RO6958688, AFM11, MT110/AMG 110, MT111/AMG211/MEDI-565, AMG330, MT112/BAY2010112, MOR209/ES414, MGD006/S80880, MGD007, and FBTA05.

8. The chimeric fusion receptor of claim 5, wherein:

(i) the ectodomain comprises a full or partial length of an extracellular portion of NKG2C, or any functional variants thereof; and

(ii) the selected agonist has a binding specificity to the ectodomain of NKG2C; or wherein the selected agonist comprises at least one of an NKG2C-IL15-CD33 TriKE, an NKG2C-IL15-CD19 TriKE, and an NKG2C-IL15-CD20 TriKE.

9. The chimeric fusion receptor of claim 5, wherein:

(i) the ectodomain comprises a full or partial length of an extracellular portion of CD28, or any functional variants thereof; and

(ii) the selected agonist has a binding specificity to the ectodomain of CD28; or wherein the selected agonist comprises at least one of 15E8, CD28.2, CD28.6, YTH913.12, 37.51, 9D7 (TGN1412), 5.11A1, ANC28.1/5D10, and 37407.

10. The chimeric fusion receptor of claim 5, wherein:

(i) the ectodomain comprises a full or partial length of an extracellular portion of CD16, CD64, or any functional variants or combined/chimeric forms thereof;

(ii) the selected agonist has a binding specificity to the ectodomain of CD16 or CD64; or wherein the selected agonist comprises at least one of IgG antibody, CD16×CD30, CD64×CD30, CD16×BCMA, CD64×BCMA, CD16-IL-EpCAM or CD64-IL-EpCAM, CD16-IL-CD33 and CD64-IL-CD33; and wherein the IL comprises all or a portion of at least one cytokine comprising IL2, IL4, IL6, IL7, IL9, IL10, IL11, IL12, IL15, IL18, IL21, or any functional variants or chimeric forms thereof.

11. The chimeric fusion receptor of any one of claims 1-10, wherein the transmembrane domain of the CFR:

(i) has no ER retention or endocytosis signals, or has ER retention and endocytosis signals removed by engineering; and

(ii) comprises all or a part of a transmembrane domain of:

(a) a transmembrane protein or a membrane protein;

(b) a protein comprising CD3 ϵ , CD3 γ , CD3 δ , CD3 ζ , CD4, CD8, CD8a, CD8b, CD27, CD28, CD40, CD84, CD137, CD166, Fc ϵ RI γ , 4-1BB, OX40, ICOS, ICAM-1, CTLA-4, PD-1, LAG-3, 2B4, BTLA, CD16, IL7, IL12, IL15, KIR2DL4, KIR2DS1, NKp30, NKp44, NKp46, NKG2C, NKG2D, a T cell receptor, a nicotinic acetylcholine receptor, a GABA receptor, or a combination thereof; or

(c) CD28, CD8, CD3 ϵ , or CD4.

12. The chimeric fusion receptor of claim 1, wherein the endodomain comprises at least a cytotoxicity domain, and optionally one or more of a co-stimulatory domain, a persistency signaling domain, a death-inducing signaling domain, a tumor cell control signaling domain, and any combinations thereof.

13. The chimeric fusion receptor of claim 12, wherein the endodomain comprises a cytotoxicity domain comprising at least a full length or a portion of CD3 ζ , 2B4, DAP10, DAP12, DNAM1, CD137 (4-1BB), IL21, IL7, IL12, IL15, NKp30, NKp44, NKp46, NKG2C, or NKG2D polypeptide; and optionally wherein the endodomain further comprises one or more of:

(i) a co-stimulatory domain comprising a full length or a portion of CD2, CD27, CD28, CD40L, 4-1BB, OX40, ICOS, PD-1, LAG-3, 2B4, BTLA, DAP10, DAP12, CTLA-4, or NKG2D polypeptide, or any combination thereof;

(ii) a co-stimulatory domain comprising a full length or a portion of CD28, 4-1BB, CD27, CD40L, ICOS, CD2, or any combination thereof;

(iii) a persistency signaling domain comprising a full length or a portion of an endodomain of a cytokine receptor comprising IL2R, IL7R, IL15R, IL18R, IL12R, IL23R, or any combination thereof; and/or

(iv) a full or a partial intracellular portion of a receptor tyrosine kinase (RTK), a tumor necrosis factor receptor (TNFR), an EGFR or a FAS receptor.

14. A cell or a population thereof, wherein the cell comprises a polynucleotide encoding the chimeric fusion receptor (CFR) of any one of ~~the~~ claims 1-13, wherein the cell is an eukaryotic cell, an animal cell, a human cell, an immune cell, a feeder cell, an induced pluripotent stem cell (iPSC), a clonal iPSC, or an derivative iPSC-derived effector cell ~~thereof~~.

15. The cell or population thereof of claim 14, wherein the effector cell further comprises one or more of:

- (i) a CAR having a targeting specificity;
- (ii) a CD16 or a variant thereof;
- (iii) CD38 knockout;
- (iv) a partial or full peptide of a cell surface expressed exogenous cytokine and/or a receptor thereof;
- (v) HLA-I deficiency, and optionally HLA-II deficiency;
- (vi) introduction of HLA-G or non-cleavable HLA-G, or knockout of one or both of CD58 and CD54;
- (vii) at least one of the genotypes listed in Table 1;
- (viii) deletion or disruption of at least one of B2M, TAP1, TAP2, Tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR, NKG2A, NKG2D, CD25, CD69, CD44, CD56, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, and TIGIT; or
- (ix) introduction of at least one of HLA-E, 4-1BBL, CD3, CD4, CD8, CD16, CD47, CD113, CD131, CD137, CD80, PDL1, A_{2A}R, antigen-specific TCR, Fc receptor, an antibody or functional variant or fragment thereof, a checkpoint inhibitor, an engager, and surface triggering receptor for coupling with an agonist.

16. The cell or population thereof of claim 15, wherein the cell has therapeutic properties comprising one or more of:
- (i) increased cytotoxicity;
 - (ii) improved persistency and/or survival;
 - (iii) enhanced ability in migrating, and/or activating or recruiting bystander immune cells, to tumor sites;
 - (iv) improved tumor penetration;
 - (v) enhanced ability to reduce tumor immunosuppression;
 - (vi) improved ability in rescuing tumor antigen escape;
 - (vii) controlled apoptosis;
 - (viii) enhanced or acquired ADCC; and
 - (ix) ability to avoid fratricide,
- in comparison to its counterpart primary cell obtained from peripheral blood, umbilical cord blood, or any other donor tissues.
17. The cell or population thereof of claim 15, wherein the CD16 or a variant thereof comprises at least one of:
- (a) a high affinity non-cleavable CD16 (hnCD16);
 - (b) F176V and S197P in ectodomain domain of CD16;
 - (c) a full or partial ectodomain originated from CD64;
 - (d) a non-native (or non-CD16) transmembrane domain;
 - (e) a non-native (or non-CD16) intracellular domain;
 - (f) a non-native (or non-CD16) signaling domain;
 - (g) a non-native stimulatory domain; and
 - (h) transmembrane, signaling, and stimulatory domains that are not originated from CD16, and are originated from a same or different polypeptide.
18. The cell of population thereof of claim 15, wherein the CAR is:
- (i) T cell specific or NK cell specific;
 - (ii) a bi-specific antigen binding CAR;
 - (iii) a switchable CAR;
 - (iv) a dimerized CAR;

- (v) a split CAR;
- (vi) a multi-chain CAR;
- (vii) an inducible CAR;
- (viii) co-expressed with a partial or full peptide of a cell surface expressed exogenous cytokine and/or a receptor thereof, optionally in separate constructs or in a bi-cistronic construct;
- (ix) co-expressed with a checkpoint inhibitor, optionally in separate constructs or in a bi-cistronic construct;
- (x) specific to at least one of CD19, BCMA, CD20, CD22, CD38, CD123, HER2, CD52, EGFR, GD2, MICA/B, MSLN, VEGF-R2, PSMA and PDL1; and/or
- (xi) specific to any one of ADGRE2, carbonic anhydrase IX (CAIX), CCR1, CCR4, carcinoembryonic antigen (CEA), CD3, CD5, CD7, CD8, CD10, CD20, CD22, CD30, CD33, CD34, CD38, CD41, CD44, CD44V6, CD49f, CD56, CD70, CD74, CD99, CD123, CD133, CD138, CDS, CLEC12A, an antigen of a cytomegalovirus (CMV) infected cell, epithelial glycoprotein-2 (EGP-2), epithelial glycoprotein-40 (EGP-40), epithelial cell adhesion molecule (EpCAM), EGFRvIII, receptor tyrosine-protein kinases erb- B2,3,4, EGFR, EGFR-VIII, ERBB folate-binding protein (FBP), fetal acetylcholine receptor (AChR), folate receptor- α , Ganglioside G2 (GD2), Ganglioside G3 (GD3), human Epidermal Growth Factor Receptor 2 (HER2), human telomerase reverse transcriptase (hTERT), ICAM-1, Integrin B7, Interleukin-13 receptor subunit alpha-2 (IL-13R α 2), κ -light chain, kinase insert domain receptor (KDR), Lewis A (CA19.9), Lewis Y (LeY), L1 cell adhesion molecule (L1-CAM), LILRB2, melanoma antigen family A 1 (MAGE-A1), MICA/B, Mucin 1 (Muc-1), Mucin 16 (Muc-16), Mesothelin (MSLN), NKCSI, NKG2D ligands, c-Met, cancer-testis antigen NY-ESO-1, oncofetal antigen (h5T4), PRAME, prostate stem cell antigen (PSCA), PRAME prostate-specific membrane antigen (PSMA), tumor-associated glycoprotein 72 (TAG-72), TIM-3, TRBC1, TRBC2, vascular endothelial growth factor R2 (VEGF-R2), Wilms tumor protein (WT-1), and a pathogen antigen; and

optionally wherein the CAR of any one of (i) to (xi) is inserted at a TCR locus, and/or is driven by an endogenous promoter of TCR, and/or the TCR is knocked out by the CAR insertion.

19. The cell or population thereof of claim 15, wherein the cell comprises a partial or full peptide of a cell surface expressed exogenous cytokine and/or a receptor thereof, and wherein the exogenous cytokine or receptor thereof:

- (a) comprises at least one of IL2, IL4, IL6, IL7, IL9, IL10, IL11, IL12, IL15, IL18, IL21, and its respective receptor(s); or
- (b) comprises at least one of:
 - (i) co-expression of IL15 and IL15R α by using a self-cleaving peptide;
 - (ii) a fusion protein of IL15 and IL15R α ;
 - (iii) an IL15/IL15R α fusion protein with intracellular domain of IL15R α truncated or eliminated;
 - (iv) a fusion protein of IL15 and membrane bound Sushi domain of IL15R α ;
 - (v) a fusion protein of IL15 and IL15R β ;
 - (vi) a fusion protein of IL15 and common receptor γ C, wherein the common receptor γ C is native or modified; and
 - (vii) a homodimer of IL15R β ,
 wherein any one of (i)-(vii) can be co-expressed with a CAR in separate constructs or in a bi-cistronic construct;
- and optionally,
- (c) is transiently expressed.

20. The cell or population thereof of claim 15, wherein the checkpoint inhibitor is an antagonist to one or more checkpoint molecules comprising PD-1, PDL-1, TIM-3, TIGIT, LAG-3, CTLA-4, 2B4, 4-1BB, 4-1BBL, A_{2A}R, BATE, BTLA, CD39, CD47, CD73, CD94, CD96, CD160, CD200, CD200R, CD274, CEACAM1, CSF-1R, Foxp1, GARP, HVEM, IDO, EDO, TDO, LAIR-1, MICA/B, NR4A2, MAFB, OCT-2, Rara (retinoic acid receptor alpha), TLR3, VISTA, NKG2A/HLA-E, and inhibitory KIR; or

wherein the engager comprises a bi-specific T cell engager (BiTE) or a tri-specific killer cell engager (TriKE).

21. The cell or population thereof of claim 15, wherein the derivative effector cell is capable of recruiting, and/or migrating T cells to tumor sites, and wherein the derivative effector cell is capable of reducing tumor immunosuppression in the presence of one or more checkpoint inhibitors.
22. The cell or population thereof of claim 14, wherein the cell comprises:
 - (i) one or more exogenous polynucleotides integrated in a safe harbor locus or a selected gene locus; or
 - (ii) more than two exogenous polynucleotides integrated in different safe harbor loci or two or more selected gene loci.
23. The cell or population thereof of claim 22, wherein the safe harbor locus comprises at least one of AAVS1, CCR5, ROSA26, collagen, HTRP, H11, GAPDH, or RUNX1; and wherein the selected gene locus is one of B2M, TAP1, TAP2, Tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR, NKG2A, NKG2D, CD38, CD25, CD69, CD44, CD58, CD54, CD56, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, or TIGIT; and/or wherein the integration of the exogenous polynucleotides knocks out expression of the gene in the locus.
24. The cell or population thereof of claim 23, wherein the TCR locus is a constant region of TCR alpha and/or TCR beta.
25. The cell or population thereof of claim 14, wherein the derivative effector cell comprises a derivative CD34 cell, a derivative hematopoietic stem and progenitor cell, a derivative hematopoietic multipotent progenitor cell, a derivative T cell progenitor, a derivative NK cell progenitor, a derivative T lineage cell, a derivative NKT lineage cell, a derivative NK lineage cell, a derivative B lineage cell, or a derivative immune effector cell having one or more functional features that are not present in a counterpart primary T, NK, NKT, and/or B cell.
26. A composition comprising the cell or population thereof of any one of the claims 14-25.
27. A master cell bank (MCB) comprising the clonal iPSC of any one of the claims 14-25.
28. A composition ~~for therapeutic use~~ comprising the cell or population thereof of any one of the claims 14-25, and one or more therapeutic agents for use in therapy.

29. The composition for use of claim 28, wherein the therapeutic agents comprise a peptide, a cytokine, a checkpoint inhibitor, an antibody or functional variant or fragment thereof, an engager, a mitogen, a growth factor, a small RNA, a dsRNA (double stranded RNA), mononuclear blood cells, feeder cells, feeder cell components or replacement factors thereof, a vector comprising one or more polynucleic acids of interest, a chemotherapeutic agent or a radioactive moiety, or an immunomodulatory drug (IMiD).

30. The composition for use of claim 29, wherein:

(a) the checkpoint inhibitor comprises:

(i) one or more antagonist checkpoint molecules comprising PD-1, PDL-1, TIM-3, TIGIT, LAG-3, CTLA-4, 2B4, 4-1BB, 4-1BBL, A₂AR, BATE, BTLA, CD39, CD47, CD73, CD94, CD96, CD160, CD200, CD200R, CD274, CEACAM1, CSF-1R, Foxp1, GARP, HVEM, IDO, EDO, TDO, LAIR-1, MICA/B, NR4A2, MAFB, OCT-2, Rara (retinoic acid receptor alpha), TLR3, VISTA, NKG2A/HLA-E, or inhibitory KIR;

(ii) one or more of atezolizumab, avelumab, durvalumab, ipilimumab, IPH4102, IPH43, IPH33, lirimumab, monalizumab, nivolumab, pembrolizumab, and their derivatives or functional equivalents; or

(iii) at least one of atezolizumab, nivolumab, and pembrolizumab; or

(b) the therapeutic agents comprise one or more of venetoclax, azacitidine, and pomalidomide; or

(c) the engager comprises a bi-specific T cell engager (BiTE), or a tri-specific killer cell engager (TriKE).

31. The composition for use of claim 29, wherein the antibody, or functional variant or fragment thereof comprises:

(a) anti-CD20, anti-CD22, anti-HER2, anti-CD52, anti-EGFR, anti-CD123, anti-GD2, anti-PDL1, and/or anti-CD38 antibody;

(b) one or more of rituximab, veltuzumab, ofatumumab, ublituximab, ocaratuzumab, obinutuzumab, ibritumomab, ocrelizumab, inotuzumab, moxetumomab, epratuzumab, trastuzumab, pertuzumab, alemtuzumab, cetuximab, dinutuximab, avelumab, daratumumab, isatuximab, MOR202, 7G3, CSL362, elotuzumab, and their humanized or Fc modified variants or fragments and their functional equivalents and biosimilars; or

(c) daratumumab, and wherein the derivative effector cell comprises a CD38 knockout, and optionally an expression of CD16 or a variant thereof.

32. ~~Therapeutic use of the~~ Use of a composition comprising the cell or population thereof of any one of the claims 14-25, and one or more therapeutic agents in the manufacture of a medicament for of any one of the claims 28-31 by introducing the composition to treating a subject suitable for adoptive cell therapy, wherein the subject has an autoimmune disorder, a hematological malignancy, a solid tumor, cancer, or a viral infection.

33. A method of manufacturing a derivative effector cell comprising the CFR of any one of claims 1-13, wherein the method comprises differentiating a genetically engineered iPSC, wherein the iPSC comprises a polynucleotide encoding the CFR, and optionally one or more edits resulting in:

- (i) CD38 knockout;
- (ii) HLA-I deficiency, and optionally HLA-II deficiency;
- (iii) introduction of HLA-G or non-cleavable HLA-G, or knockout of one or both of CD58 and CD54;
- (iv) a CD16 or a variant thereof;
- (v) a CAR having a targeting specificity;
- (vi) a partial or full peptide of a cell surface expressed exogenous cytokine and/or a receptor thereof;
- (vii) at least one of the genotypes listed in Table 1;
- (viii) deletion or disruption of at least one of B2M, TAP1, TAP2, Tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR, NKG2A, NKG2D, CD25, CD69, CD44, CD56, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, and TIGIT; or
- (ix) introduction of at least one of HLA-E, 4-1BBL, CD3, CD4, CD8, CD16, CD47, CD113, CD131, CD137, CD80, PDL1, A_{2A}R, antigen-specific TCR, Fc receptor, an antibody or functional variant or fragment thereof, a checkpoint inhibitor, an engager, and surface triggering receptor for coupling with an agonist.

34. The method of claim 33, further comprising genomically engineering a clonal iPSC to knock in a polynucleotide encoding the CFR; and optionally:

- (i) to knock out CD38,
 - (ii) to knock out B2M and/or CIITA,
 - (iii) to knock out one or both CD58 and CD54, and/or
 - (iv) to introduce HLA-G or non-cleavable HLA-G, a high affinity non-cleavable CD16 or a variant thereof, a CAR, and/or a partial or full peptide of a cell surface expressed exogenous cytokine and/or a receptor thereof.
35. The method of claim 34, wherein the genomic engineering comprises targeted editing.
36. The method of claim 35, wherein the targeted editing comprises deletion, insertion, or in/del, and wherein the targeted editing is carried out by CRISPR, ZFN, TALEN, homing nuclease, homology recombination, or any other functional variation of these methods.
37. CRISPR mediated editing of clonal iPSCs, wherein the editing comprises a knock-in of a polynucleotide encoding the CFR of any one of claims 1-13.
38. The CRISPR mediated editing of claim 37:
- (a) wherein the editing of clonal iPSCs further comprises knocking out TCR, or
 - (b) wherein the CFR is inserted at one of the gene loci comprising: B2M, TAP1, TAP2, Tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR, NKG2A, NKG2D, CD38, CD25, CD69, CD44, CD58, CD54, CD56, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, or TIGIT; and wherein the insertion knocks out expression of the gene in the locus.
39. A ~~method of composition~~ comprising cells comprising the CFR of any one of claims 1-13 and an agonist specific to the CFR for use in therapy, wherein the composition is to be administered to a subject in need thereof ~~treating a disease or a condition comprising administering to a subject in need thereof cells comprising the CFR of any one of claims 1-13 and an agonist specific to the CFR.~~
40. The ~~method~~ composition for use of claim 39, wherein the cells comprising the CFR express an antibody or functional variant or fragment thereof, or an engager that is specific to the CFR.

41. The ~~method~~ composition for use of claim 39, wherein the cells comprising the CFR are iPSC-derived effector cells further comprising one or more of:

- (i) a CD38 knockout;
- (ii) TCR^{neg};
- (iii) an exogenous CD16 or a variant thereof;
- (iv) HLA-I and/or HLA-II deficiency;
- (v) introduction of HLA-G or non-cleavable HLA-G, or knockout of one or both of CD58 and CD54;
- (vi) introduction of a CAR; and/or
- (vii) a partial or full peptide of a cell surface expressed exogenous cytokine and/or a receptor thereof.

42. The ~~method~~ composition for use of claim 39, wherein administration of the cells results in one or more of:

- (i) increased cytotoxicity;
- (ii) improved persistency and/or survival;
- (iii) enhanced ability in migrating, and/or activating or recruiting bystander immune cells, to tumor sites;
- (iv) improved tumor penetration;
- (v) enhanced ability to reduce tumor immunosuppression;
- (vi) improved ability in rescuing tumor antigen escape;
- (vii) controlled apoptosis;
- (viii) enhanced or acquired ADCC; and
- (ix) ability to avoid fratricide,

in comparison to their counterpart primary cells obtained from peripheral blood, umbilical cord blood, or any other donor tissues.